90%, ref. 1) than that of TxB_2 (approx. 30%, this paper). Thus a decrease in PGE_2 metabolism to 75% would lead to a 2.5-fold increase in PGE_2 survival from 10% to 25% whereas for TxB_2, a similar decrease in metabolism would increase survival by a factor of 1.3 (from 70% to 95%). Our method of collecting 3 sec fractions may not be of sufficient resolution to detect the smaller change in TxB_2 efflux kinetics.

In other studies with ANTU-treated lung, the metabolism of 5-hydroxytryptamine [8] and that of AMP was decreased [9] but no efflux measurements were reported in either study. In the absence of any direct estimates we would speculate that the pharmacokinetics of $PGF_{2\alpha}$, a substrate metabolised as extensively as PGE_2 in rat lung [10], would respond as do those of PGE_2 to ANTU treatment.

In summary, the pharmacokinetics of PGE_2 and sucrose in lungs taken from rats were altered by the oedema induced *in vivo* by ANTU, but the magnitude of the effect on PGE_2 kinetics was much greater than that on sucrose. As sucrose is a marker for extracellular volume, this result showed that the slower efflux of PGE_2 was not merely due to an increase in extracellular volume in oedematous lung. Furthermore since the efflux of radioactivity derived from TxB_2 was not affected by ANTU treatment, although its metabolism was inhibited, PGE_2 appears to be the best substrate so far tested to demonstrate the pharmacokinetic effects of this type of pulmonary oedema.

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REFERENCES

1. Y. S. Bakhle, *Biochem. Pharmac.* **31**, 3395 (1982).
2. Y. S. Bakhle, A. M. Reynard and J. R. Vane, *Nature* **222**, 956 (1969).
3. A. L. A. Boura and R. D. Murphy, *Clin. exp. Pharmac. Physiol.* **5**, 387 (1978).
4. T. M. Fitzpatrick, L. S. Friedman, P. A. Kot and P. W. Ramwell, *Br. J. Pharmac.* **70**, 295 (1980).
5. K. Patel and Y. S. Bakhle, *Prostaglandins, Leukotrienes and Medicine* **10**, 221 (1983).
6. T. E. Eling, H. J. Hawkins and M. W. Anderson, *Prostaglandins* **14**, 51 (1977).
7. C. Robinson, S. H. Peers, K. A. Waddell, I. A. Blair and J. R. S. Hoult, *Biochim. biophys. Acta* **712**, 315 (1982).
8. E. R. Block and F. J. Schoen, *Am. Rev. resp. Dis.* **123**, 69 (1981).
9. Y. S. Bakhle and C. J. Grantham, *J. Physiol.* **365**, 106P (1985).
10. M. W. Anderson and T. E. Eling, *Prostaglandins* **11**, 645 (1976).

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Marked inhibition of histamine formation in transplantable histamine-producing gastric carcinoid of *Mastomys natalensis* by (*S*)-α-fluoromethylhistidine and its potent antiulcer effect on tumor-bearing hosts

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(*S*)-α-Fluoromethylhistidine (FMH) is a potent and specific inhibitor of L-histidine decarboxylase (HDC, L-histidine carboxy-lyase, EC 4.1.1.22) and strongly inhibits histamine formation *in vitro* and *in vivo* [1–3].

Matsomys natalensis, an African rodent ranging in size between a mouse and a rat, is the only mammal other than humans to develop gastric carcinoid at a high incidence [4, 5]. Our previous studies showed that gastric carcinoids, either primary or transplantable, contained large amounts of histamine and revealed appreciable HDC activity [6–8]. The most conspicuous effect of gastric carcinoid on the host is the development of severe duodenal ulcer(s) due to the hypersecretion of gastric acid evoked by histamine released from a growing tumor [9].

It seems important to elucidate the *in vivo* effect of FMH on histamine formation in a growing transplanted tumor and the ulceration in the duodenum of the host.

Materials and methods

Mastomys used, tumor transplanation, urine collection and determinations of histamine in urine and tumor tissues have been described in detail in previous reports [6–9]. The transplantable tumor strain producing large amounts of histamine belongs to strain B as described in a previous study [9], and six male *Mastomys*, each bearing a growing transplant in the 12th generation, were used. Three animals from one litter were untreated, and three from another litter were treated with FMH. FMH was donated by Dr. J. Kollonitsch of Merck Sharp & Dohme Research Laboratories, Rahway, NJ, U.S.A. It was administered by either daily intraperitoneal (i.p) injection (100 mg/kg) or continuous subcutaneous (s.c.) infusion (100 mg/kg/24 hr) through an Alzet osmotic minipump (model 2001, Alza Corp., Palo Alto, CA, U.S.A.), as used in a previous experiment [10].

Results

In the present experiment, the transplanted tumors were palpable 4 months after transplantation. The urinary histamine levels (normal level: 0.56 ± 0.15 μg/24 hr [8]) in the untreated group paralleled the tumor growth (Fig. 1A). When the animals became sluggish (sign preceding perforating duodenal ulcer), they were killed, their duodena were inspected, and tumor histamine concentrations were measured. The duodena were enormously distended and were accompanied by multiple ulcers (Fig. 2A). The histamine concentration in the tumor tissues of three animals (mean ± S.E.) was 98.4 ± 10.2 μg/g wet tissue (range, 75.8 to 118.7 μg).

On the other hand, when the tumor-bearing *Mastomys* were given FMH by daily i.p. injections, their urinary histamine levels fell 20% below the preinjection level (Fig. 1B). Three days after termination of FMH injections, i.e. day 13 of observation, the tumor-bearing *Mastomys* excreted about three times more histamine in urine than on day 1 of observation. On the next day, the osmotic minipumps filled with FMH were implanted subcutaneously in the dorsal region of the same animals, and continuous infusion of FMH was initiated. The histamine levels in urine were more drastically lowered to 6, 8 and 8% of

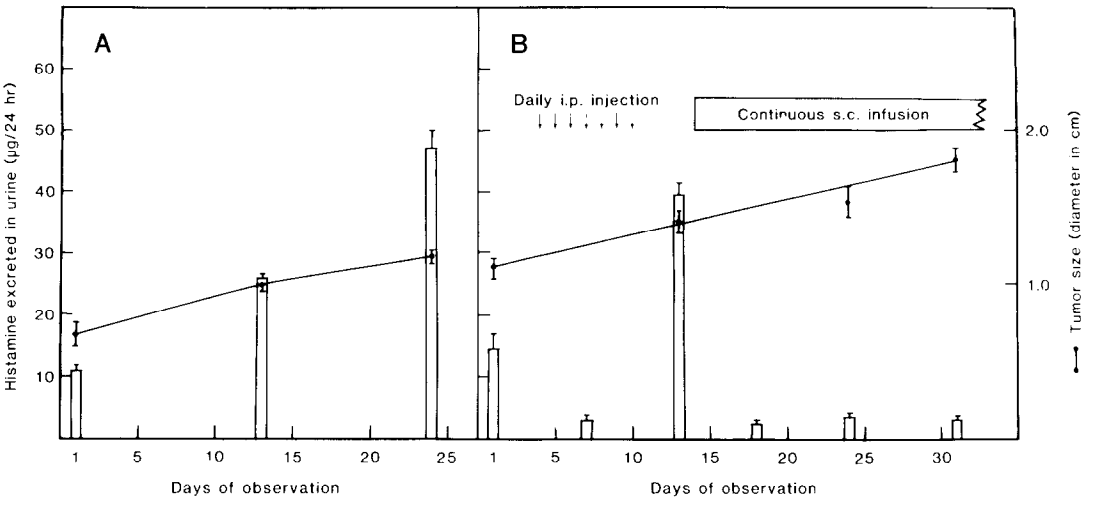

Fig. 1. Urinary histamine levels (histogram) and tumor sizes (●—●) in untreated (A) and FMH-treated (B) tumor-bearing *Mastomys*. Each value represents the mean ± S.E. of three tumor-bearing animals. The tumor size of each *Mastomys* was first expressed as the mean of its largest and smallest diameters, and the mean ± S.E. of three tumors was calculated.

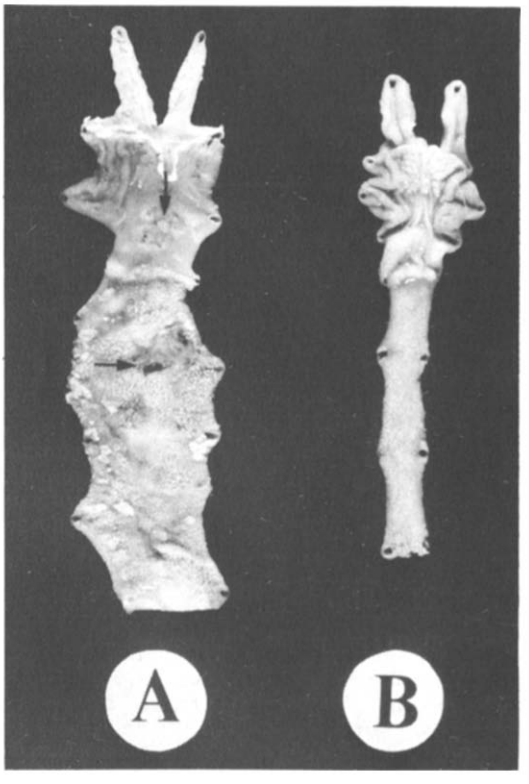

Fig. 2. Stomachs and duodena of tumor-bearing *Mastomys* after opening along the greater curvature (stomach) and the posterior wall (duodenum). (A) Organs from an untreated animal killed on day 42 of observation. The duodenum is greatly distended. The horizontal arrow indicates the largest and perforated ulcer among multiple ulcers developing in the duodenum. This animal also developed a gastric ulcer in the lesser curvature (vertical arrow). (B) organs from an animal treated with FMH, as indicated in Fig. 1B, and killed on day 43. The stomach and duodenum are of normal size. The mucosa is smooth without ulceration.

the level before FMH infusion on day 18, 24 and 31 of observation respectively. All three tumor-bearing *Mastomys* were killed on day 43 of observation in order to examine duodenal ulceration and to determine the histamine concentration in tumor tissues. The duodena of three animals were not distended, and their mucosal surfaces were smooth with no ulceration (Fig. 2B). The tumor histamine concentration in the three animals (mean ± S.E.) was 3.3 ± 0.5 μg/g wet tissue (range, 2.3 to 4.1 μg).

Discussion

The marked suppressive effect of FMH on histamine formation *in vivo* was also confirmed in the present transplantable gastric carcinoid of *Mastomys* selected as a suitable model, since the urinary histamine levels, of which the major portion is produced in the transplanted tumor, were decreased drastically by either daily i.p. injection or continuous s.c. infusion (Fig. 1B), and the histamine concentrations in the tumor tisues were also greatly decreased by the latter treatment. It is worth noting that the continuous infusion of FMH was more effective for suppressing the urinary histamine levels than the i.p. injection of equal FMH doses (100 mg/kg/24 hr), indicating the advantage of constant administration of a small amount of FMH for inhibiting intracellular HDC activity in the present carcinoid cells (Fig. 1B).

In addition, the suppression of histamine excreted in urine was maintained at almost the same level for the entire period of FMH infusion, regardless of concomitant tumor growth (Fig. 1B). In this respect, FMH does not appear to retard the growth of this tumor strain unlike other transplantable neoplastic cells in mice and rats [11].

Of particular interest is the fact that FMH suppressed the formation of severe duodenal ulcers which invariably develop in the host bearing a growing transplant and excreting large amounts of histamine. It is apparent that this antiulcer effect of FMH results from the sequential events, including inhibition of tumor HDC activity, lowered histamine concentrations in tumor and blood, and suppressed acid secretion from gastric parietal cells. The previous study gave concrete evidence that *Mastomys* is more susceptible to the induction of duodenal ulcer(s) by exogeneous histamine than other rodent species such as mouse and rat [10]. The human stomach is also susceptible to gastric acid secretion by histamine as a secretagogue [10], and ample

clinical and experimental evidence for the importance of histamine in the development of duodenal ulcer disease has been presented since the introduction of H_2-receptor antagonists [12]. Therefore, FMH may well be useful for the treatment of certain kinds of duodenal ulcer disease associated with high HDC activity in humans.

In summary, FMH administered by either i.p. injection or continuous s.c. infusion leads to marked depletion of histamine levels in urine of *Mastomys* bearing transplantable histamine-producing argyrophilic gastric carcinoid. The continuous s.c. infusion of FMH appears to be superior to i.p. injection for suppressing HDC activity in carcinoid cells. The most conspicuous effect of FMH used *in vivo* on tumor-bearing hosts is to suppress the duodenal ulcer(s) that invariably develops in untreated animals.

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REFERENCES

1. J. Kollonitsch, A. A. Patchett, S. Marburg, A. L. Maycock, L. M. Perkins, G. A. Doldouras, D. E. Duggan and S. D. Aster, *Nature, Lond.* **274**, 906 (1978).
2. K. Macyama, T. Watanabe, A. Yamatodani, Y. Taguchi, H. Kambe and H. Wada, *J. Neurochem.* **41**, 128 (1983).
3. D. E. Duggan, K. F. Hooke and A. L. Maycock, *Biochem. Pharmac.* **33**, 4003 (1984).
4. K. C. Snell and H. L. Stewart, *Science* **163**, 470 (1969).
5. K. C. Snell and H. L. Stewart, *Gann Monogr.* **8**, 39 (1969).
6. S. Hosoda, W. Nakamura, K. C. Snell and H. L. Stewart, *Science* **170**, 454 (1970).
7. S. Hosoda, W. Nakamura, K. C. Snell and H. L. Stewart, *Biochem. Pharmac.* **20**, 2671 (1971).
8. S. Hosoda, T. Saito, H. Suzuki and K. Hara, *S. Afr. Cancer Bull.* **24**, 394 (1980).
9. M. Suyama, T. Saito, K. Hara, H. Kito and S. Hosoda, *J. natn Cancer Inst.* **72**, 751 (1984).
10. S. Hosoda, H. Ikedo and T. Saito, *Gastroenterology* **80**, 16 (1981).
11. J. Bartholeyns and M. Bouclier, *Cancer Res.* **44**, 639 (1984).
12. C. J. Wood and M. A. Simkins (Eds.), *International Symposium on Histamine H_2-Receptor Antagonist.* Smith, Kline & French, London (1973).

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Effect of ethanol on inhibition of striatal adenylate cyclase activity

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The activity of adenylate cyclase is under dual regulation coupled to both stimulatory and inhibitory drug receptors [see Refs. 1 and 2]. Addition of a stimulatory drug, such as glucagon, isoproterenol or dopamine, in combination with a short-chain alcohol results in a synergistic activation of adenylate cyclase [3–5]. Although short-chain alcohols also increase basal adenylate cyclase activity [3–5], this increase is only partly responsible for the alcohol-induced increase in dopamine-stimulated activity in the striatum [4]. Furthermore, the presence of dopamine increases the sensitivity of adenylate cyclase to ethanol [4].

Striatal adenylate cyclase is also coupled to opiate and muscarinic receptors, but activation of these receptors attenuates enzymatic activity [6–10]. Short-chain alcohols have been reported to decrease the affinity of muscarinic and opiate receptors for agonists [11–15], but the effects of alcohol on drug-induced inhibition of adenylate cyclase have not been investigated. The present study was, therefore, undertaken to determine the *in vitro* effects of ethanol on the inhibition of striatal adenylate cyclase by morphine, Leu-enkephalin and acetylcholine.

Methods

Female Holtzmann rats were killed by decapitation and the striata rapidly removed. Tissues were homogenized (Brinkmann Polytron) in 2 mM Tris–HCl (pH 7.5) containing 2 mM ethyleneglycolbis(amino-ethylether)tetraacetate (EGTA), and the homogenates were centrifuged at 20,000 *g* for 15 min at 4°. The resulting pellets after resuspension in the Tris–EGTA buffer were again centrifuged, and this procedure was repeated. The final pellet, which was used to measure adenylate cyclase activity, was resuspended in Tris–EGTA (2.2 to 3.9 mg protein/ml)

Adenylate cyclase activity was determined by measuring the conversion of [α-^{32}P]ATP to [^{32}P]cyclic AMP. Reactions were carried out in a final volume of 150 μl containing 50 mM Tris–HCl (pH 7.5), 5 mM cyclic AMP, 2 mM $MgCl_2$, 50 μM GTP, 1 mM theophylline, 100 mM NaCl, 0.1 mM ATP (containing 1–2 million cpm of [α-^{32}P]ATP), 10 mM creatine phosphate, 0.1 mg/ml creatine kinase and various drugs. Eserine (10 μM) was included in all samples containing acetylcholine. Reactions were carried out for 10 min at 30° and were terminated by the addition of 100 μl of a solution containing 50 mM Tris–HCl (pH 7.5), 5 mM ATP and 10% sodium dodecyl sulfate. After incubating the tubes for 15 min in boiling water, sample volumes were increased to 1 ml with water, and a modification [5] of the method of Salomon *et al.* [16] was used to isolate the [^{32}P] cyclic AMP. Protein content was measured by the method of Lowry *et al.* [17] using bovine serum albumin (Fraction V) as a standard. Data were analyzed for statistical significance using Dunnett's test for multiple comparisons [18].

Results and discussion

Addition of ethanol elicited a linear, dose-related increase in adenylate cyclase activity in both the presence and absence of inhibitory drug (Fig. 1). Although stimulatory drugs have been shown previously to increase the sensitivity of adenylate cyclase to activation by ethanol