REFERENCES

1. H. BRIEGER and J. TEISINGER (Eds.), *Proc. Int. Symp. Toxicology of Carbon Disulfide*, Prague 1966. Excerpta Medica Foundation (1967).
2. L. S. DJERASSI and R. LUMBROSO, *Br. J. ind. Med.* **25,** 220 (1968).
3. A. E. COHEN and L. D. SCHEEL, *Am. ind. Hyg. Assn. J.* **20,** 303 (1959).
4. B. SOUCEK, *J. Hyg. Epidem. microbiol. Immun.* **1,** 10 (1957).
5. T. GESSNER and M. JAKUBOWSKI, *Pharmacologist* **12,** 272 (1970).
6. A. MUSIL and K. IRGOLIC, *Z. analyt. Chem.* **208,** 352 (1965).
7. K. ROTHWELL and R. L. WAIN, *Ann. appl. Biol.* **51,** 161 (1963).
8. T. GESSNER and M. JAKUBOWSKI, *Biochem. Pharmac.* **21,** 219 (1972).
9. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
10. C. N. REMY, *J. biol. Chem.* **238,** 1078 (1963).
11. J. BREMER and D. M. GREENBERG, *Biochim. biophys. Acta* **46,** 217 (1961).

Biochemical Pharmacology, Vol. 21, pp. 3076–3078. Pergamon Press, 1972. Printed in Great Britain.

Action of some *N*-methyl derivatives of histamine on salivary and lacrimal secretion of the cat

(*Received* 9 *May* 1972; *accepted* 24 *May* 1972)

IN A previous study one of us[1] investigated the action of a series of histamine derivatives methylated in the side chain amino group, on vascular and extravascular smooth muscle preparations: it was shown that histamine was the most potent compound and that the histaminic activity decreased by increasing the number of methyl groups: taking the activity of histamine as 100 the monomethyl derivative (MMH) was 60–95 per cent as potent and the dimethyl derivative (DMH) 20–55 per cent as potent, the trimethyl derivative (TMH) had a very poor histaminic activity but showed a marked "nicotinic" action. In other investigations it was found that monomethyl and especially dimethyl-histamine, which were found to occur in nature, were actually more active than histamine in stimulating gastric secretion in dogs,[2–4] cats[5] and in the guinea pig.[6]

We wanted to investigate whether other exocrine secretions like salivary and lacrimal secretions behaved as the gastric one or as the smooth muscle.

Experiments were performed on the anaesthetized cat: salivary secretion was measured with a drop counter after cannulation of the submaxillary duct; lacrimal secretion was evaluated by the Schirmer's technique.[7] A simultaneous recording of the systemic blood pressure and respiration was always taken to check continuously the general conditions of the animals.

Compounds used were histamine, the two natural methyl derivatives *N'*-methyl-histamine [4-(2-methylaminoethyl)imidazole] and *N'N'*-dimethyl-histamine [4-(2-dimethylaminoethyl)imidazole], and *N'N'N'*-trimethyl-histamine [4-(2-trimethylaminoethyl)imidazole] which is the quaternary ammonium base of histamine hitherto unknown in nature. Each compound was injected intravenously.

As far as salivary secretion is concerned results are summarized in Fig. 1. It is evident from the figure that mono and dimethyl derivatives behaved exactly as histamine and had quite parallel dose–response curves. Their activities however, were less pronounced than that of the mother substance. The maximum effect was rather low for the three compounds, probably owing to the hypotensive activity which was very remarkable at the highest doses tested (500–750 μg/kg). The behaviour of the trimethyl derivative was quite different and the maximum effect was approximately three times as high as that of histamine. The dose–response curve of this substance paralleled that of nicotine tested for comparison. Nicotine, however, was less active both as regards potency (of which the main index is represented by the threshold dose) and efficacy (of which the main index is represented by the maximum effect).[8] Trimethyl-histamine and especially nicotine caused a remarkable hypertensive effect and a short lasting stimulation of respiratory activity.

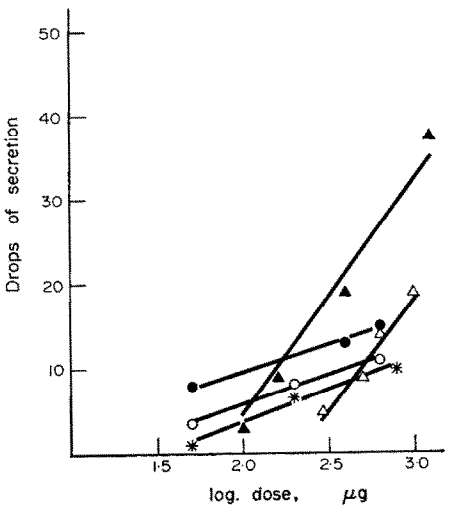

FIG. 1. Effect of histamine methyl histamines and nicotine on the salivary secretion of the anaesthetized cat. On the ordinate drops of secretion; on the abscissa log of doses (μg/kg) administered. Each point represents the mean of the values obtained from three to six animals. ●—● = histamine; ○—○ = MMH; *—* = DMH; ▲—▲ = TMH; △—△ = nicotine.

As to lacrimal glands we could not determine the complete dose–response curves for each compound because of the progressive decrease of lacrimal secretion evident in each experiment, both in regard to basal and to stimulated secretion. For this reason the potency of the different compounds was expressed in terms of ratio of activity obtained at doses 2–3 times higher than the threshold dose (see Table 1). It appears from the table that mono- and dimethyl histamine were less active than

TABLE 1. EFFECT OF HISTAMINE, METHYL HISTAMINES AND NICOTINE ON THE LACRIMAL SECRETION OF THE ANEASTHETIZED CAT (SCHIRMER'S TEST)

Compounds	Number of animals	Effect
Histamine	6	100
Monomethyl histamine	5	60·5 ± 14·1
Dimethyl histamine	4	45·6 ± 19·4
Trimethyl histamine	6	141·0 ± 16·5
Nicotine	6	88·2 ± 11·0

The effect of histamine was arbitrarily taken as 100. The effect of other substances was calculated as mean per cent effect (±S.E.) in comparison with that of histamine.

histamine which in its turn was approximately half as active as TMH. The maximum effect of histamine, MMH and DMH was rather low since the increase of secretion in comparison with basal levels, never reached values higher than 250–300 per cent. On the contrary the maximum effect of TMH was extremely pronounced and the maximal dose (1 mg/kg) provoked an increase of +1000 per cent in comparison with basal secretion. The strong effect of TMH both on salivary and lacrimal secretion is not surprising, taking into account the importance of "nicotinic" effect on salivary and lacrimal glands. Indeed in our experimental conditions the action of TMH was only slightly inhibited by mepiramine but almost completely blocked by hexamethonium and this confirmed the major role of nicotinic receptors in the mechanism of action of this quaternary ammonium base. The effect of nicotine was similar but less intense.

Since in preliminary experiments we showed (Bertaccini and Impicciatore, unpublished) that also in stimulating pancreatic secretion the two naturally occurring methyl derivatives (MMH and DMH) are less potent than the mother substance, it may be suggested that gastric glands are probably the only area in which these *N*-methyl histamines are more potent than histamine at least in some animal species.

Acknowledgement—This work was supported by a grant from the Consiglio Nazionale delle Ricerche, Roma.

Departments of Pharmacology and Pharmaceutical Chemistry, University of Parma, Parma, Italy

G. BERTACCINI
M. IMPICCIATORE
F. MOSSINI

REFERENCES

1. G. BERTACCINI and T. VITALI, *J. Pharm. Pharmac.* **16,** 441 (1964).
2. T. M. LIN, R. S. ALPHIN, F. G. HENDERSON, D. N. BENSLAY and K. K. CHEN, *Ann. N.Y. Acad. Sci.* **99,** 30 (1962).
3. H. NAVERT, E. V. FLOCK, G. M. TYCE and C. F. CODE, *Physiologist* **12,** 313 (1969).
4. G. BERTACCINI, M. IMPICCIATORE and F. MOSSINI, *Congr. Soc. Ted. Ital. Pharmac.* Heidelberg, Sept. 1970.
5. C. F. CODE, S. M. MASLINSKI, F. MOSSINI and H. NAVERT, *J. Physiol.* **217,** 557 (1971).
6. E. M. KOVACS and S. HEISLER, *Eur. J. Pharmac.* **11,** 361 (1970).
7. O. SCHIRMER, *Albrecht v. Graefes Arch. Ophthalm.* **56,** 497 (1903).
8. M. I. GROSSMAN, *Proceedings of the Symposium on Origin, Chemistry, Physiology, and Pathophysiology of Gastrointestinal Hormones* Wilsbaden, Munich, Schattauer (1969).

Biochemical Pharmacology, Vol. 21, pp. 3078–3082. Pergamon Press, 1972. Printed in Great Britain.

Kininase activity in equine plasma

(*Received* 10 *January* 1972; *accepted* 24 *May* 1972)

THE KININOGENS and kallikreins derived from horse plasma have been thoroughly studied by Henriques *et al.* [1–5] No information is available, however, concerning the kininases of this plasma. Many reports have been published describing the kininases of other species, including humans.[6–10] This paper describes some of the characteristics of equine plasma kininases and compares them with the plasma kininases of the rat and guinea-pig.

Materials and methods

Bradykinin acetate was synthesized by the Peptide Center, Institute for Protein Research, Osaka University, Osaka, Japan; 2-bromo-*d*-lysergic acid diethylamide (BOL 148) and 1-methyl-lysergic acid butanolamide (UML 491), were kindly supplied by Sandoz Ltd., Basle, Switzerland; tranexamic acid was supplied by Daiichi Seiyaku Co., Ltd., Tokyo, Japan; 1,10-phenanthroline, was purchased from Wako Pure Chemical Industries, Ltd., Tokyo, Japan; soyabean trypsin inhibitor (SBTI), from Worthington Biochemical Co., U.S.A., hexesterol, from Teikoku Hormone Mfg. Co., Ltd., Tokyo, Japan and heparin sodium, from Sigma, Mo, U.S.A.

Plasma was used as the source of plasma kininases. Blood samples were drawn by venepuncture from the external jugular vein of unanaesthetized male thoroughbred horses, 4–9 years old, and