Brit. J. Pharmacol. (1964), **22**, 520–526.

EFFECTS OF CHLORPROMAZINE AND BROMOLYSERGIC ACID DIETHYLAMIDE ON GASTRIC SECRETION OF ACID INDUCED BY HISTAMINE IN RATS

BY

B. O. AMURE* AND M. GINSBURG

From the Department of Pharmacology, University of Bristol

(*Received December 5*, 1963)

In anaesthetized rats in which the lumen of the stomach was perfused with 0.001 to 0.00025 N-sodium hydroxide solution and the *p*H of effluent fluid was recorded continuously, intravenous administration of chlorpromazine caused transient inhibition of acid secretion. After acid secretion had returned to the control level the responses to histamine were greater than those before chlorpromazine was given. Aminoguanidine, iproniazid and bromolysergic acid diethylamide also potentiated the effect of histamine on acid secretion but the initial inhibition was absent. Indirect evidence from experiments in which mixtures of aminoguanidine with chlorpromazine or bromolysergic acid diethylamide and of iproniazid with chlorpromazine or bromolysergic acid diethylamide were given, suggests that chlorpromazine and bromolysergic acid diethylamide enhance responses to histamine by inhibition of imidazole-*N*-methyl transferase.

Known pathways for histamine catabolism are oxidative deamination by diamine oxidase and methylation in the imidazole ring (Rothschild & Schayer, 1958). In man, imidazole ring methylation is the most important pathway, and 50 to 70% of the radioactivity of injected [^{14}C]-histamine appears in the urine as methylated derivatives (Schayer & Cooper, 1956 ; Nilsson, Lindell, Schayer & Westling, 1959 ; Lindell, Nilsson, Roos & Westling, 1960). The enzyme, imidazole-*N*-methyl transferase, which catalyses the methylation of histamine in the imidazole ring to give 1-methyl-4-(β-aminoethyl)imidazole (methylhistamine) is inhibited *in vitro* by chlorpromazine, bromolysergic acid diethylamide and by cupric ions (Brown, Tomchick & Axelrod, 1959 ; Lindahl, 1960). In experiments on the cat, White (1961) perfused the cerebral ventricles with [^{14}C]-histamine and found that previous treatment of the cat with chlorpromazine reduced the proportions of labelled methyl imidazole derivatives in the brain and in the perfusate ; he suggested that the action of chlorpromazine might be due to inhibition of the histamine methylating enzyme.

The present experiments were undertaken to demonstrate the effects of imidazole-*N*-methyl transferase inhibitors on responses to histamine *in vivo*. It was anticipated that high doses of chlorpromazine might be required and, in order to avoid complications arising from the slight antihistamine action of the drug, the effect of histamine on gastric acid secretion was selected as the response for study, since this is not

* Present address: Department of Physiology, University of Ibadan, Nigeria.

inhibited by antihistamines. Furthermore, the experiments of Ghosh & Schild (1958), who demonstrated potentiation of the effect of histamine on gastric acid secretion in rats by diamine oxidase inhibitors, suggested that this response was suitable for our purpose.

METHODS

Albino rats, weighing 150 to 350 g, were used. Male animals were chosen because Westling (1958) has shown that in male rats more exogenous histamine is methylated than in female animals. The rats were anaesthetized with urethane (0.6 ml./100 g intramuscularly of a 25% solution) and were prepared for perfusion of the stomach as described by Ghosh & Schild (1958). Sodium hydroxide solution (0.001 to 0.00025 N) was perfused through the stomach at the rate of 1 ml./min ; the gastric effluent fluid was passed through a flow glass electrode (E.I.L. Type SMF 23), and *p*H was recorded continuously on an ink-writing recorder. The dead space between the acid secreting area of the stomach and the glass electrode was approximately 0.5 ml. Injections were given intravenously through a cannulated external jugular vein in volumes of 0.05 to 0.2 ml.

The following drugs were used: chlorpromazine hydrochloride (Largactil, May & Baker); bromolysergic acid diethylamide (Sandoz Products); iproniazid (Marsilid, Roche Products); nialimide (Niamid, Pfizer); aminoguanidine carbonate (L. Light & Co.) and histamine dihydrochloride (L. Light & Co.). Doses refer to the salts, except for histamine, where doses refer to the base.

RESULTS

The response to histamine

The gastric acid secretory response of rats to intravenous histamine, described by Ghosh & Schild (1958), was confirmed in all respects. After 5 to 7 min from injection of the drug, the *p*H of the gastric effluent fluid began to fall and reached the lowest value in 13 to 15 min ; recovery to the control level followed within 30 to 50 min. Injections were given slowly over 15 to 20 sec, and were tolerated without evidence of respiratory embarrassment. The doses of histamine required to elicit responses varied widely ; in some animals effects were seen after the injection of 5.5 μg of histamine, in others as much as 110 μg was ineffective.

Effect of chlorpromazine and bromolysergic acid

Responses to histamine given before, and from 2 to 15 min after, intravenous injection of chlorpromazine (0.5 mg/100 g) are shown in Fig. 1. In some experiments the *p*H of the gastric effluent fluid rose sharply immediately after the chlorpromazine was given. This inhibition of acid secretion was transient and the *p*H of the effluent fluid returned to the preinjection level within 15 min. Histamine was given after the inhibitory effect of chlorpromazine had passed or, if inhibition did not occur, histamine was given 2 min after the dose of chlorpromazine. The responses to histamine injected after chlorpromazine were bigger and lasted longer than those obtained before chlorpromazine was given but the latency of the response was unaltered. The potentiating effect of chlorpromazine was relatively short-lived: responses to histamine given 1 hr after chlorpromazine were no greater than before chlorpromazine. Bromolysergic acid diethylamide (0.05 mg/100 g) also enhanced responses to histamine but the initial transient rise in the *p*H of the gastric effluent fluid, observed with chlorpromazine, was absent.

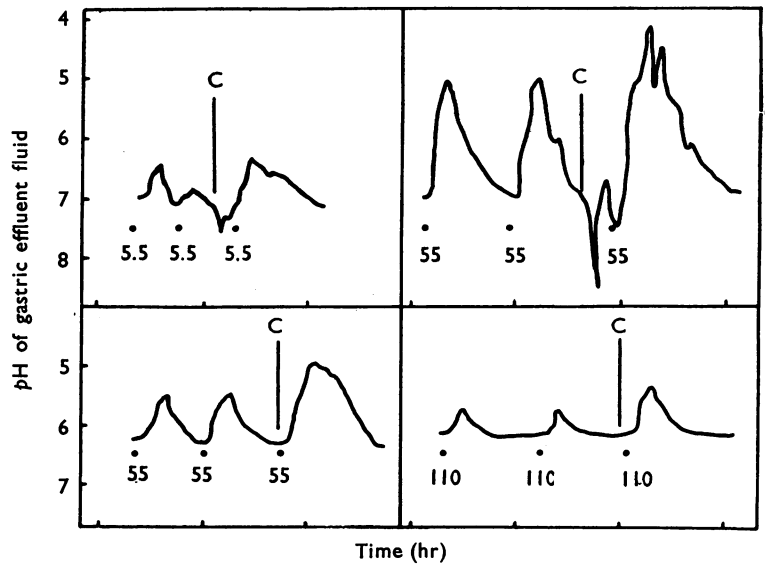

Fig. 1. Effects of histamine on *p*H of gastric secretion in anaesthetized rats before and after intravenous injection of chlorpromazine (at C, 0.5 mg/100 g). Histamine was injected at the black dots in the doses (μg) shown. Abscissae: time scale in hours.

Effect of chlorpromazine after aminoguanidine

Ghosh & Schild (1958) showed that in rats the response to histamine was enhanced after treatment with aminoguanidine. In the experiment illustrated in Fig. 2 responses to histamine were tested after injections of 0.5 and 0.7 mg of aminoguanidine; histamine was given 2 min after each dose of the diamine oxidase inhibitor. After the first injection of aminoguanidine the response to histamine was enhanced, 11 μg of histamine giving a bigger response that had been previously obtained with 22 μg. A second injection of aminoguanidine did not further enhance the response to histamine, the effect of 5.5 μg of histamine being smaller than the effect of the previous injection of 11 μg of histamine. However, when 1.5 mg of chlorpromazine was then given together with 0.5 mg of aminoguanidine, the response to 5.5 μg of histamine, given 2 min later, was strikingly potentiated. After an interval of 1 hr the dose of aminoguanidine was repeated, this time without chlorpromazine, and the response to 5.5 μg of histamine was now similar to that obtained before chlorpromazine had been injected.

Since increasing the total dose of aminoguanidine did not enhance responses to histamine further, it was concluded that maximum inhibition of diamine oxidase had been attained; the capacity of chlorpromazine to produce further potentiation of the response shows that its action is distinct from that of aminoguanidine.

Effect of chlorpromazine or bromolysergic acid together with iproniazid

Monamine oxidase inhibitors, iproniazid and nialimide, potentiate gastric acid secreting responses to histamine and interfere with the metabolism of histamine

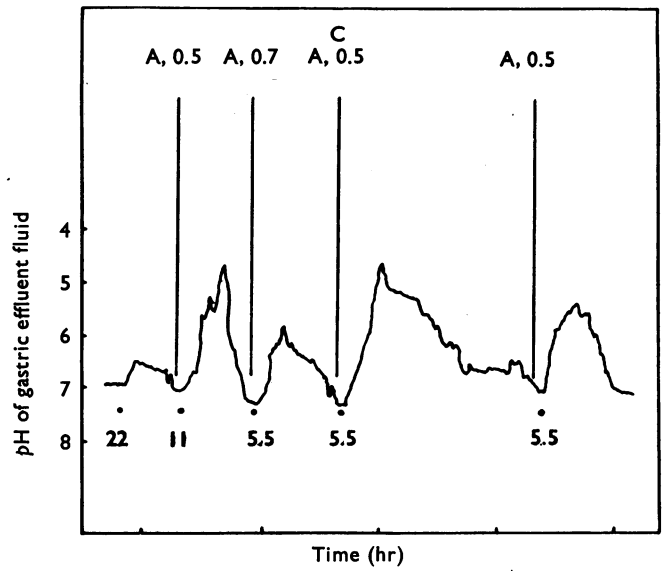

Fig. 2. Effect of chlorpromazine (at C, 1.5 mg, simultaneously with aminoguanidine) on *p*H of gastric secretion induced by histamine in an anaesthetized rat treated with aminoguanidine (at A, doses in mg shown). Histamine was injected at the black dots in the doses shown (μg). Abscissa: time scale in hours.

by inhibiting the oxidation of ring-methyl histamine to methyl imidazole acetic acid (Schayer, 1959). Fig. 3 illustrates experiments similar in design to that of Fig. 2. Iproniazid was given in increasing doses until no further enhancement of the response to histamine was observed. When this state was reached, chlorpromazine or bromolysergic acid diethylamide was given together with another dose of iproniazid ; the response to histamine given 2 min later was greater than that obtained with iproniazid alone. It was concluded, therefore, that enhancement of responses to histamine by chlorpromazine was independent of the enhancement produced by iproniazid.

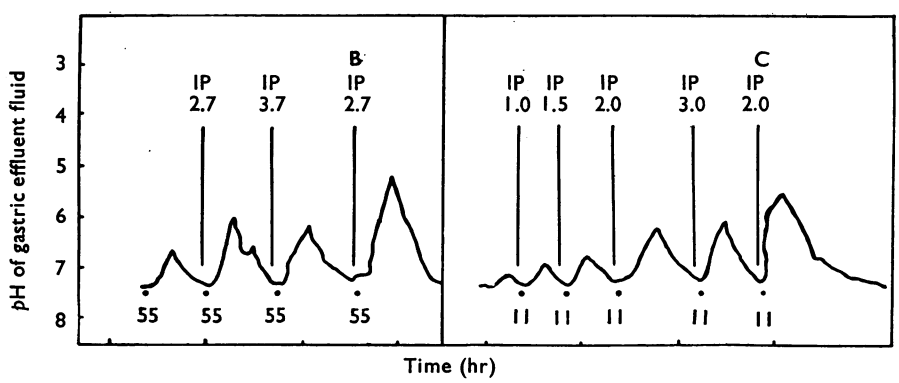

Fig. 3. Effects of chlorpromazine (at C, 1.0 mg) and bromolysergic acid diethylamide (at B, 0.25 mg) on *p*H of gastric secretion induced by histamine (at black dots, doses in μg) in anaesthetized rats treated with iproniazid (at IP, doses in μg). Abscissa: time scale in hours.

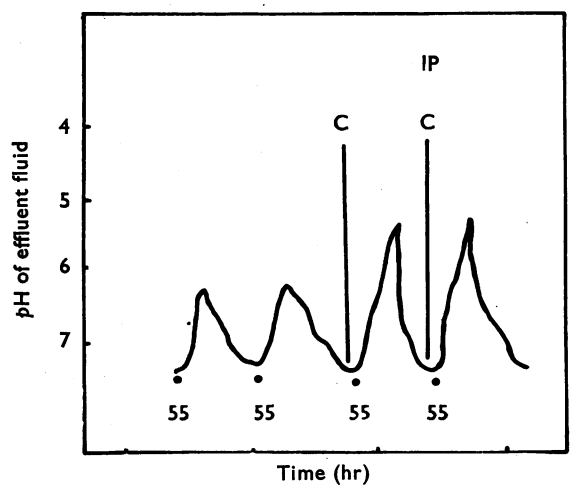

Fig. 4. Effect of iproniazid (at IP, 3 mg) on *p*H of gastric secretion induced by histamine (at black dots, doses in μg) in an anaesthetized rat treated with chlorpromazine (at C, 0.5 mg). Abscissa: time scale in hours.

In the experiment illustrated in Fig. 4 the response to histamine (55 μg) was enhanced by the previous injection of chlorpromazine (0.5 mg) but, when the dose of histamine was repeated after the same dose of chlorpromazine given together with iproniazid (3 mg), no further potentiation was observed.

Effect of aminoguanidine in combination with iproniazid

Since chlorpromazine enhanced the effect of histamine on acid secretion after iproniazid or aminoguanidine had produced maximum potentiation, it was concluded

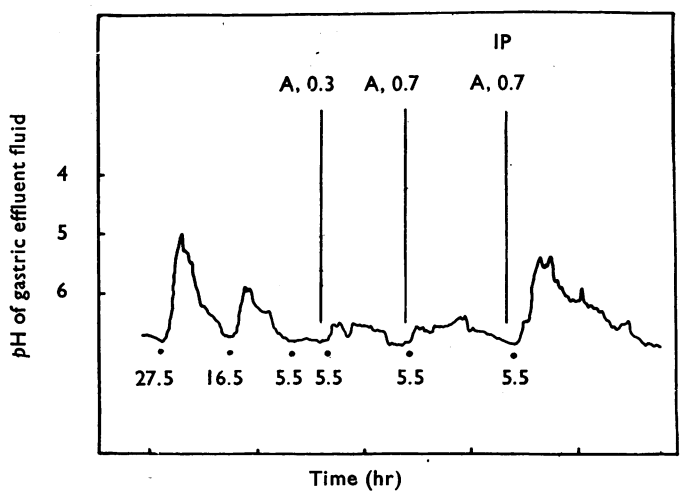

Fig. 5. Effect of iproniazid (at IP, 1.5 mg) on *p*H of gastric secretion induced by histamine (at black dots, doses in μg) in an anaesthetized rat treated with aminoguanidine (at A, doses in mg). Abscissa: time scale in hours.

that chlorpromazine differed in action from the monamine oxidase and diamine oxidase inhibitors. The validity of this conclusion was tested in the experiment illustrated in Fig. 5 in which iproniazid was given to an animal in which aminoguanidine had produced its full potentiating effect.

Aminoguanidine in a dose of 0.3 mg, enhanced the response to 5.5 μg of histamine which, previously, had been hardly discernible. A subsequent injection of aminoguanidine (0.7 mg) did not further potentiate the response to histamine but, when given together with 1.5 mg of iproniazid, histamine gave an enhanced response which was now almost as large as that obtained with five times the dose of histamine before treatment with the inhibitors.

DISCUSSION

In some of the rats a sharp and transient rise in the *p*H of the gastric effluent fluid was observed after treatment with chlorpromazine (Fig. 1). This effect may be analogous to the inhibition of gastric acid secretion by chlorpromazine which has been demonstrated in healthy human subjects (Haverbach, Stevenson, Sjoerdsma & Terry, 1955), in patients with duodenal ulcer (Sun & Shay, 1959a), in dogs (Sun & Shay, 1959b) and in rats (Tiede, Pfeiffer & Gass, 1963 ; Konturek & Radecki, 1963). The effect is probably of central origin and mediated by the vagus (Sun & Shay, 1959b). However, after intravenous administration to anaesthetized animals, the duration of the inhibition of gastric secretion by chlorpromazine is short (5 to 15 min) and, when control levels of acid secretion are restored, enhanced responses to histamine can be demonstrated.

The evidence presented in this paper shows that potentiation of the response to histamine by chlorpromazine is distinguishable from the similar effects of diamine oxidase and monamine oxidase inhibitors. Indirect evidence of the mode of action of chlorpromazine is given by the observations that chlorpromazine further enhances responses to histamine in animals previously treated with iproniazid, whereas additional potentiation of responses to histamine does not occur when iproniazid is given to animals previously treated with chlorpromazine. In animals treated with iproniazid alone, in which oxidative deamination of methylhistamine by monamine oxidase is inhibited (Rothschild & Schayer, 1958), exogenous histamine and its ring-methylated derivative will persist at higher concentrations than in untreated animals ; if chlorpromazine inhibits formation of methylhistamine, even greater potentiation of the effect of histamine would be expected to occur in animals previously treated with iproniazid. However, when chlorpromazine is given alone, the effect of histamine is potentiated but the subsequent administration of iproniazid does not affect responses to histamine, probably because there is less substrate for monamine oxidase.

We are indebted to the Medical Research Council for support of this work by a grant to one of us (B.O.A.).

REFERENCES

BROWN, D. D., TOMCHICK, R. & AXELROD, J. (1959). The distribution and properties of a histamine-methylating enzyme. *J. biol. Chem.*, **234**, 2948–2950.

GHOSH, M. N. & SCHILD, H. O. (1958). Continuous recording of acid gastric secretion in the rat. *Brit. J. Pharmacol.*, **13**, 54–61.

HAVERBACH, B. J., STEVENSON, T. D., SJOERDSMA, A. & TERRY, L. L. (1955). The effects of reserpine and chlorpromazine on gastric secretion. *Amer. J. med. Sci.*, **230**, 601–604.

KONTUREK, S. & RADECKI, T. (1963). The influence of phenothiazine derivatives on gastric secretion and the development of peptic ulcers. *Biochem. Pharmacol.*, **12**, suppl. 134.

LINDAHL, K. M. (1960). The histamine methylating enzyme system in liver. *Acta physiol. scand.*, **49**, 114–138.

LINDELL, S. E., NILSSON, K., ROOS, B. E. & WESTLING, H. (1960). The effect of enzyme inhibitors on histamine catabolism in man. *Brit. J. Pharmacol.*, **15**, 351–355.

NILSSON, K., LINDELL, S. E., SCHAYER, R. W. & WESTLING, H. (1959). Metabolism of C^{14}-labelled histamine in pregnant and non-pregnant women. *Clin. Sci.*, **18**, 313–319.

ROTHSCHILD, Z. & SCHAYER, R. W. (1958). Synthesis and metabolism of a histamine metabolite, 1-methyl-4-(β-aminoethyl)-imidazole. *Biochim. biophys. Acta (Amst.)*, **30**, 23–27.

SCHAYER, R. W. (1959). Catabolism of physiological quantities of histamine in vivo. *Physiol. Rev.*, **39**, 116–126.

SCHAYER, R. W. & COOPER, J. A. D. (1956). Metabolism of C^{14}-histamine in man. *J. appl. Physiol.*, **9**, 481–483.

SUN, D. C. H. & SHAY, H. (1959a). Synergistic action of chlorpromazine and Tricyclamol on basal gastric secretion in duodenal ulcer patients. *Gastroenterology*, **36**, 245–250.

SUN, D. C. H. & SHAY, H. (1959b). Mechanism for the inhibition of gastric secretion by chlorpromazine in the dog. *J. Pharmacol. exp. Ther.*, **126**, 155–157.

TIEDE, R. N., PFEIFFER, C. J. & GASS, G. H. (1963). Reduction by chlorpromazine of pylorus-ligation ulcers in the rat. *Nature (Lond.)*, **199**, 1296.

WESTLING, H. (1958). The difference in the metabolism of injected [^{14}C]-histamine in male and female rats. *Brit. J. Pharmacol.*, **13**, 498–500.

WHITE, T. (1961). Inhibition of the methylation of histamine in cat brain. *J. Physiol. (Lond.)*, **159**, 191–197.