EFFECT OF AMODIAQUINE ON GASTRIC HISTAMINE METHYLTRANSFERASE AND ON HISTAMINE-STIMULATED GASTRIC SECRETION

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1 Amodiaquine was found to be a potent inhibitor in vitro of gastric histamine methyltransferase from human and canine corpus and from pig antrum. The ID$_{50}$ for the enzyme, purified from pig antrum mucosa by ultracentrifugation and chromatography on DEAE-cellulose, was 2.5 $\mu$M.

2 In six dogs with Heidenhain pouches the maximum secretory response to histamine (40 $\mu$g/kg i.m.) was augmented by i.m. injection of amodiaquine. The augmentation depended on the dose of amodiaquine, the optimum effect (40% increase in volume of gastric juice, 80% in acid output) being achieved with 2 mg/kg. The maximum secretory response to betazole was also enhanced by amodiaquine.

3 It was suggested that amodiaquine may enhance the histamine and betazole stimulated gastric secretion by an inhibition of gastric histamine methyltransferase in vivo.

Introduction

The stimulation of gastric acid secretion by pharmacological doses of histamine, the occurrence of histamine both in gastric juice and tissue and the release of histamine by gastrin in the rat gastric mucosa predominantly support the hypothesis that histamine is a physiological stimulator of gastric secretion (Code, 1956; Kahlson, Rosengren, Svahn & Thunberg, 1964; Lorenz & Pfleger, 1968). Although this hypothesis has been rejected by several authors (Grossman, 1967; Johnson, 1971), others have continued to defend the idea that histamine may be the common mediator for the action of gastrin and acetylcholine on the parietal cell (Code, Maśliński, Mossini & Navert, 1971; Kahlson & Rosengren, 1971). These differences in opinion were based on disagreements about subordinate elements in the hypothesis: adequate localization of histamine in the mucosa (pro: Thunberg, 1967; contra: Håkanson, 1970), release of histamine during physiological stimulation of acid secretion (pro: Kahlson et al., 1964; contra: Håkanson, 1970) and formation of histamine in the gastric mucosa of various mammalian species (pro: Kahlson et al., 1964; Lorenz, Halbach, Gerant & Werle, 1969; contra: Aures, Davidson & Håkanson, 1969).

However, there is good agreement about the inactivation of histamine in the stomach. High activities of histamine methyltransferase (S-adenosyl-methionine: histamine-N-methyltransferase; EC 2.1.1.8) were demonstrated in man and all animals investigated (Brown, Tomchick & Axelrod, 1959; Lorenz, Barth & Werle, 1970a), but no, or only a very low, activity of diamine oxidase (diamine: oxygen-oxidoreductase EC 1.4.3.6) could be measured in the gastric mucosa of various mammals, including rats, dogs and cows (Kusche, Lorenz, Hahn & Werle, 1969; for a review see Lorenz et al., 1970a).

Therefore we attempted to find a potent inhibitor of gastric histamine methyltransferase in vitro and then to investigate its effect on histamine-stimulated gastric secretion; the antimalarial drug amodiaquine was already known to be an inhibitor of this enzyme from the rat kidney (Cohn, 1965). A preliminary report of our findings has been published (Troidl, Lorenz, Barth, Seidel, Rohde, Goecke, Schmal & Hamelmann, 1972).
Methods

Source and preparation of histamine methyltransferase

Gastric mucosa from duodenal ulcer patients (treated by a Billroth II gastrectomy), from dogs (laboratory animals) or from pigs (slaughter house) was rinsed with ice-cold Tyrode solution immediately after removal of the stomach. The mucosa was stripped off from the muscular layer, frozen with dry ice and then stored at -20°C for several days.

Crude extracts of histamine methyltransferase were prepared by homogenizing the frozen tissues with 2 vol 0.25 M sucrose solution. The homogenate was centrifuged at 2°C and 100,000 x g for 30 min and the supernatant was used as the source of the enzyme. Histamine methyltransferase from pig antrum mucosa was partially purified by applying the crude extract to a column of DEAE-cellulose (Lorenz, Reimann, Barth, Kusche, Meyer, Doenicke & Hutzel, 1972).

The fractions containing the highest enzyme activities were combined and used directly for incubation. The purification of this enzyme preparation was 22-fold as compared with the crude homogenate, the specific activity being 4.04 nmol/(min x mg protein) with a yield of 74%. The proteins were determined according to Lowry, Rosebrough, Farr & Randall (1951).

Reagents

Histamine dihydrochloride puriss. (Fluka, Buchs); 2,5-diphenyloxazole (POPOP), 2,2'-p-phenylene-bis-(5-phenyloxazole) (POPOP) and toluene (all scintillation grade), isoamylalcohol p.a., D(+)-sucrose puriss., nicotinamide p.a., Folin-Ciocalteu's phenol reagent, 0.1 N sodium hydroxide solution (Titrisol), all inorganic salts, acids and bases p.a. (Merck, Darmstadt); S-adenosyl-L-[14C]-methylmethionine (0.51 mCi/mmole) (The Radiochemical Centre, Amersham); S-adenosyl-L-methionine hydrogen sulphate (Boehringer, Mannheim); amodiaquine (kindly supplied by R. Wolf, Parke & Davies, Munich); DEAE-cellulose-SS p.a. (Serva, Heidelberg). All solutions were prepared with twice-distilled water.

Drugs

Sterile solutions of histamine dihydrochloride and amodiaquine were prepared by the University's pharmacy; betazole dihydrochloride (Histalog, Lilly, Giessen); insulin (Hoechst, Frankfurt).

Cannula for the Heidenhain pouch experiments

The experiments were planned to last many months and so, to minimize the risk of technical failure, a special tissue-sparing corrosion-resistant cannula was developed based on the approved models of Dragstedt, Haymond & Ellis (1933), Gregory (1950) and DeVito & Harkins (1959). This cannula consisted of a stainless steel tube placed on a shaft of polypropylene. A relatively broad plexiglass disk, pushed over the stainless steel tube, was fixed on one side by the polypropylene shaft and on the other side by the interior surface of the abdominal wall (Emás, Swan & Jacobson, 1967). The disk was intended to shunt off the strong pulling and shearing forces which act occasionally on the exterior part of the cannula in the lying animal and so prevent the cannula from breaking out of the pouch.

Determination of histamine methyltransferase activity in vitro

The enzyme activity was determined by the isotope assay of Barth, Lorenz & Niemeyer (1973a) using S-adenosyl-L-[14C-methyl]-methionine as the methyl-group donor. Amodiaquine, dissolved in 0.1 ml of 0.05M sodium phosphate buffer at pH 7.4, was added to the incubation mixture, which contained 32-48 nmol histamine, 250 nmol S-adenosyl-L-methionine, 100 nmol S-adenosyl-L-[methyl 14C]-methionine and 22.5 μmol phosphate buffer, pH 7.4. The final volume of the incubation mixture was 0.5 ml. The incubation was stopped after 0, 15, 30, 45 and 60 min by the addition of borate buffer, pH 10.0.

The reaction product, [14C]-1-(r)-methylhistamine, was extracted into a mixture of toluene and isoamylalcohol. After the addition of scintillant, an aliquot was counted in a Tri Carb liquid scintillation spectrometer. The formation of 1 nmol of 1-methylhistamine corresponded to 114 ct/minute. The observed specific activities of histamine methyltransferase lay between 98 and 574 pmol/(min x mg protein), corresponding to counts of values of 1470-4350 ct/min over 60 minutes. Depending on incubation time, the blanks with heat-inactivated enzyme showed values of 80-150 ct/min over 60 min above the background of 30 ct/minute.

Evaluation of the effect of amodiaquine on histamine-stimulated gastric secretion in the Heidenhain pouch of dogs

The effect of amodiaquine on histamine and betazole stimulated gastric acid secretion was
investigated in six mongrel dogs (20-30 kg, both sexes) with Heidenhain pouches. The preparation of these pouches, the supervision of the dogs after the operation and during the test period, and the experimental design for the stimulation of gastric secretion by secretagogue alone or by secretagogue and amodiaquine (cf. Figure 1) in serial tests were performed as described by Troidl, Lorenz, Barth, Rohde, Feifel, Schmal, Goecke, Reimann-Huhnd & Seidel (1973).

Additionally every six months, insulin (0.2 i.u./kg) was used to check the lack of vagal reinnervation of the pouches. During the first 90 min of this test the blood sugar concentration was determined every 15 min by the glucose oxidase assay (Boehringer-Kit, Mannheim). The result of the insulin test was only considered to be satisfactory if the glucose concentration fell to values between 30 and 10 mg/100 ml blood (Baron, 1970).

The procedure for a single test was the same as described by Troidl et al. (1973), using 40 μg/kg histamine or 2 mg/kg betazole instead of pentagastrin. Gastric juice was collected in 30 min-portions for 2.5 to 3 h, after which time in all experiments the basal secretion plateau was attained. Determination of the volume and the acidity of each sample was carried out as described by Troidl et al. (1973).

Definitions

The maximum secretory response to histamine and betazole was defined by two parameters, the total secretion and the peak secretion. The total secretion consisted of all fractions of gastric juice obtained within the 2.5 to 3 h of secretion and was expressed as ml and mEq/secretion period. The peak secretion was represented by those two consecutive 30 min fractions, irrespective of time, which showed the greatest volumes and acid outputs and was expressed in ml and mEq/hour. The effect of amodiaquine was expressed as the ratio of the results of the single test with amodiaquine plus stimulant to the average results of the two control tests without amodiaquine (Troidl et al., 1973). Augmentation or inhibition of the stimulated secretion by amodiaquine was expressed as a percentage, because the variation of the secretion values was relatively high from animal to animal.

Statistical calculations

Statistical calculations (Snedecor & Cochran, 1967) including those of mean values, coefficient of variation, standard deviations and tests for statistical significance (Student's t-test or t-test for paired data) were performed with the aid of a desk computer (Olivetti Programma 102).
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Results

Inhibition of gastric histamine methyltransferase by amodiaquine in vitro

Amodiaquine was found to be a very potent inhibitor of histamine methyltransferase from human and canine corpus mucosa and from pig antrum mucosa (Figure 2). The ID$_{50}$ value using pig enzyme was smaller by one order of magnitude than found with the enzymes from man or dog. Crude and partially purified enzyme preparations were inhibited by amodiaquine to the same extent, since the ID$_{50}$ for histamine methyltransferase following chromatography on DEAE-cellulose was 2.5 µM (cf. Figure 2). A 30 min preincubation of amodiaquine and the enzyme at 37°C enhanced the inhibitory effect by 20% at inhibitor concentrations smaller than 50 µM.

Effect of amodiaquine on the histamine and betazole stimulated gastric secretion in Heidenhain pouch dogs

In six dogs, the maximum secretory response to histamine (40 µg/kg i.m.) showed an excellent reproducibility (for an example see Figure 1). The total volume was 34 ± 17 ml, the total output 3.8 ± 1.9 mEq/secretion period (x ± s.d.). Amodiaquine enhanced the total gastric secretion (Figure 3). The optimum dose was 2 mg/kg which enhanced the volume of gastric juice by 40% and the acid output by 80%. A higher dose of amodiaquine (3 mg/kg) produced a less marked increase and in three dogs, 5 mg/kg inhibited the total gastric secretion by about 50%. Weakness and apathy were observed in these dogs indicating that this dose was toxic (Lorenz, Thermann, Messmer, Schmal, Dormann, Kusche, Barth, Tauber, Hutzel, Mann & Uhlig, 1974). Amodiaquine augmented the peak secretion (24 ± 9 ml/h, 2.8 ± 1.0 mEq/h) and the total secretion to the same degree. In all
experiments, amodiaquine (2 mg/kg) enhanced the peak volume of gastric juice by 37 ± 18%, the peak acid output by 82 ± 31%. The total volume of the gastric juice increased by 36 ± 16%, the total acid output by 80 ± 33%. This indicates that histamine-stimulated gastric secretion was enhanced but not prolonged by amodiaquine. The secretion period following stimulation was 120-150 min both in experiments with histamine alone and in those using the combined stimulation by histamine plus amodiaquine.

The histamine isomer betazole, which has been used as a stimulator of human gastric secretion (Feifel, Lorenz, Heimann & Wörsching, 1972) was administered in two dogs in combination with amodiaquine. In four experiments, the maximum gastric secretion evoked by 2 mg/kg i.m. betazole (total volume 68 ml, total acid output 7.5 mEq/secretion period), was enhanced by 24 ± 10% (volume) and 45 ± 18% (acid output) after amodiaquine (2 mg/kg). The augmentation of the peak secretion by amodiaquine did not differ significantly from that of the total secretion.

However, these findings emphasized that amodiaquine was capable of inhibiting the histamine methyltransferase in vivo. Therefore, the enhancement by amodiaquine of gastric secretion in dogs stimulated by histamine, or betazole which releases histamine (Stubrin, Dyce, Brem, Tecimer & Haverback, 1965; Lorenz, Feifel, Schmal, Hutzel & Werle, 1970b) may be explained as an inhibition of the high activities of this enzyme in the fundic and corpus mucosa (Lorenz et al., 1970a).

It has previously been shown that pentagastrin-stimulated secretion is also augmented by amodiaquine (Trojdel et al., 1972, 1973). Studies on the in vivo inhibition of gastric histamine methyltransferase by agents other than amodiaquine were performed in rats by Amure & Ginsburg (1964a, b), using chlorpromazine and bromolysergic acid diethylamide and by Haverback, Stubrin & Dyce (1965), who administered 5-hydroxytryptamine and 1-(r)-methylhistamine to dogs with a total stomach gastric fistula. Both groups of authors described an augmentation of the histamine- and gastrin-stimulated acid secretion by the previously mentioned agents. But histamine methyltransferase is absent in the gastric mucosa of rats (Brown et al., 1959; Kim, Backus, Harris & Rourke, 1969), and Haverback et al. (1965) used very small doses of the drugs (0.5-1.0 μg/kg, min⁻¹), which are far below that of ID₅₀ for an inhibition of histamine methyltransferase in vitro (Brown et al., 1959). Thus it is not clear by which mechanism the drugs mentioned above enhanced gastric acid secretion.

The question whether the enhancement of the histamine-stimulated acid secretion by amodiaquine was the result of a local inhibition of histamine methyltransferase or of a general delay in histamine inactivation can partly be answered by determining the elimination-rate of exogenously administered histamine from dog’s plasma after the injection of amodiaquine. Applied in doses of 2.5 and 5.0 mg/kg this compound did not protract histamine elimination from the plasma (Lorenz et al., 1974). Furthermore, amodiaquine did not release histamine (Lorenz et al., 1974). Thus, amodiaquine seemed to enhance gastric acid secretion by inhibiting histamine methyltransferase in the glandular part of the canine mucosa, but not by influencing the concentrations of histamine in the systemic circulation.

However, explanations for the effect of amodiaquine on gastric secretion other than inhibition of gastric histamine methyltransferase, have to be considered. A direct action on the parietal cell due to calcium mobilization or to influences on the system of cyclic nucleotides can largely be

Discussion

Antimalarial drugs derived from 4-aminoquinoline were reported to be potent in vitro inhibitors of catechol-O-methyltransferase (Spector, personal communication; see Cohn, 1965) and histamine methyltransferase from rat kidney (Cohn, 1965). The ID₅₀ values were smaller by 1-3 orders of magnitude than those of other inhibitors of the latter enzyme, such as 1-(r)-methylhistamine (Brown et al., 1959; Lindahl, 1960; Barth et al., 1973a), 5-hydroxytryptamine (Brown et al., 1959; Gustafsson & Forshall, 1964), chlorpromazine and bromolysergic acid diethylamide (Brown et al., 1959), Cu²⁺-ions (Lindahl, 1960; Gustafsson & Forshall, 1964), antihistamines of the H₁-receptor type (Netter & Bodenschatz, 1967; Cohn & Wyll, 1968; Barth, Niemeyer & Lorenz, 1973b, c) and H₂-receptor antagonists (Barth et al., 1973b, c). We have now shown that amodiaquine is a potent inhibitor of histamine methyltransferase of the gastric tissue of man, dog and pig.

In vivo, investigations on the inhibition of histamine methyltransferase by amodiaquine were performed in rats by studying the daily excretion of histamine in the urine (Cohn, 1966; Kim & Cohn, 1966). The increase of the histamine excretion which was observed has to be interpreted primarily as an inhibition of the renal histamine methyltransferase, since high activities of this enzyme were found in rat kidneys.
excluded since amodiaquine does not cause acid secretion when given alone (Troidl et al., 1973). Vagal stimulation by amodiaquine seems unlikely for the same reason because prostigmine and carbachol elicited a considerable gastric secretion in the same animals in which amodiaquine was completely ineffective (Troidl et al., 1973).

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