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HISTAMINE IN THE PIG: DETERMINATION, DISTRIBUTION, RELEASE AND PHARMACOLOGICAL ACTIONS

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The specificity of the fluorometric assay of histamine was studied using three different isolation procedures. Histamine was identified by thin-layer chromatography, enzymatic degradation by diamine oxidase, fluorescence spectra and by two different biological tests. The histamine concentration of many tissues, the whole blood and plasma of young and adult pigs were determined. Especially high histamine contents were found in lung, stomach and small intestinum. The tissues of young animals contained smaller amounts of histamine than those of adult animals.

Compound 48/80 and polymyxin B, but not the detergent chremophor El, released histamine from tissues into the plasma. The gastric mucosa stored histamine, whereas some of the other tissues studied showed decreased histamine concentrations.

The pharmacological actions of histamine, betazole, serotonin and kinins on the blood pressure, and the stimulation of exocrine glands in the digestive tract by histamine were studied. Histamine and betazole caused a biphasic blood pressure response in pigs which could explain the increase of blood pressure after injection of 48/80.

Histamine, in pig Pig histamine Compound 48/80 Betazole Kinins

1. INTRODUCTION

During the last few years the pig has become increasingly important as a laboratory animal in many fields of biology and medicine (Bustad and McClellan, 1965; 1966). For example, the problems of liver transplantation were preferably studied in this species (Calne et al., 1967; 1967a; Terblanche et al., 1968; Mickaeloff and Calne, 1969). Furthermore, extracorporeal pig liver perfusion is of clinical importance in the treatment of the human acute hepatic failure (Eiseman, 1966; Abouna et al., 1968; Eiseman, 1970; Zimmermann et al., 1970). However, substances which influence circulation, smooth muscles, exocrine and endocrine glands under pharmacological and physiological conditions, like biogenic amines and different polypeptides, have rarely been studied in the pig.

Histamine, in the pig, was first detected and identified by ultimate organic analysis in the liver (Best et al., 1927; Ackermann and Fuchs, 1939). Later, the concentrations of this amine were determined in some organs of this species by biological and fluorometric assays (for a survey see Lorenz and Werle, 1969). However, no systematic studies have been performed, in the pig, on the histamine content of various organs of one and the same animal, on its relationship to age, on the chemical identification of histamine in the tissues and body fluids and on the

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release of histamine by different drugs. Only few data are known about the effects of histamine on circulation (Inchley, 1929; Smith, 1951) and on exocrine glands of the digestive tract (Bustad and McClellan, 1965). Therefore, some of these problems were studied and the results are presented in this paper.

2. MATERIALS AND METHODS

2.1. *Materials*

The tissues were obtained from adult pigs (race Deutsches Edelschwein, 100–150 kg, female) immediately after death by electrical shock in the slaughter house, and from young pigs of the same race (15–25 kg, both sexes and castrated) and guinea-pigs (400 g, female) after a blow on the head and bleeding. They were frozen by liquid nitrogen and kept at −20°C for about two weeks; whole blood and plasma were studied immediately after withdrawal.

Reagents: Histamine dihydrochloride puriss., o-phthaldialdehyde puriss. p.a. (recrystallized from ligroin p.a., b.p. 40–60°) (Fluka). Methanol, heptane, solvents for thin layer chromatrography, inorganic acids and bases (Uvasol ®), ammonium sulphate p.a. (Merck). Butanol (for chromatography, Riedel de Haën). Dowex 50 W-X 8, H^+, mesh 200–400 and DEAE cellulose-SS p.a. (Serva). Cellulose MN 300 for thin-layer chromatography (Macheray, Nagel and Co.). Sephadex G 25 (Deutsche Pharmacia), tris buffer for biochemical research, Boehringer and Soehne.

Dimetinden maleate (Fenistil ®, Zyma-Blaes). Antazoline (Antistin ®, Ciba). Heparin for biochemical research (180 I.U./mg), acetylcholine, prostigmin, (Hoffmann-La Roche). Compound 48/80 (Imperial Chemistry Industries, Manchester). Polymyxin B (Pfizer, Karlsruhe). Serotonin creatinine sulphate (Merck), bradykinin and kallidin (Sandoz). Secretin (Boots Pure Drug and Co. Ltd.). Betazole dihydrochloride (Histalog ®, Lilly). Sodium Pentobarbital (Nembutal ®, Abbott). Dextran-60 (Macrodex ®, Knoll).

2.2. *Isolation of histamine from tissues and body fluids and fluorometric assay of this amine*

2.2.1. Preparation of extracts from tissue, whole blood and plasma

Tissues were homogenized with 9 volumes of 0.4 N $HClO_4$ by an Ultraturrax homogenizer, centrifuged for 5 min at 1800 *g*; 4.0 ml of the supernatant were used for the determination of histamine. The whole blood, 5.0 ml per sample, was withdrawn with a polyethylene syringe via a polyethylene catheter in the femoral vein and mixed with 5.0 ml of 1 N $HClO_4$. After centrifugation, as described before, 4.0 ml of the supernatant were used for histamine assay.

For the preparation of plasma extracts, 19.5 ml of blood were withdrawn through the same catheter into a 20 ml polyethylene syringe containing 2 mg heparin dissolved in 0.5 ml of 0.9% NaCl solution. The content of the syringe was gently mixed without bubble formation, transferred to a 30 ml polyethylene tube, which had been cooled thoroughly by ice-cold water and centrifuged for 30 min, at 800–1000 *g* and 0–2°C. 6 ml of the plasma were mixed with 2 ml of 2 N $HClO_4$, centrifuged for 10 min at 1800 *g* and the whole supernatant filtered through a paper filter for histamine assay. The preparation of plasma extracts should be carried out in the cold as rapidly and gently as possible (Lorenz and Werle, 1969).

2.2.2. Isolation of histamine from pig tissues, guinea-pig tissues and pig plasma by cation exchange chromatography on Dowex 50 (Dowex method)

4.0 ml of the tissue extracts or the whole supernatant of the plasma treated by perchloric acid (see above) were adjusted to pH 6.5 with 2 N NaOH using a glass electrode and applied to a small column of Dowex 50 W-X 8, H^+ (0.3 × 2 cm, equilibrated with 0.1 M sodium phosphate buffer, pH 6.5). The column was in turn washed with 5.0 ml of 0.1 M sodium phosphate buffer pH 6.5, 1.0 ml of twice distilled water and 5.0 ml of 1 N HCl.

Histamine was eluted with 3.0 ml of 4 N HCl. For the fluorometric assay of the amine, the eluate was diluted with an equal volume of twice distilled water.

1.7 ml of this solution was mixed with 0.8 ml of 5 N NaOH and 0.1 ml of o-phthaldialdehyde (1% w/v, in methanol). Exactly 2 min later 0.6 ml of 2 M H_3PO_4 were added and the fluorescence measured in a Zeiss spectrofluorometer at a temperature of 21 ± 0.5°C. The excitation wavelength was 360 nm, the fluorescence wavelength 450 nm. The fluorophore was stable for at least 1 hr. The fluorescence intensity was directly proportional to the histamine concentra-

tion in a range of 1–700 ng of histamine dihydrochloride/ml of the diluted eluate. Recovery of histamine: 95–100%, coefficient of variation 4.2% (n = 18).

2.2.3. Isolation of histamine from the whole blood of pig by cation exchange chromatography on Dowex 50 followed by n-butanol extraction (combined method)

4.0 ml of the pig whole blood extract (see above) were adjusted to pH 6.5, and histamine isolated by cation exchange chromatography as described under 2.2.2. The whole undiluted eluate from the column was adjusted to pH 11.5–12.0 with 5 N NaOH using a glass electrode and shaken for 20 min in a 25 ml glass stoppered Erlenmeyer flask together with 1.5 g of solid NaCl and 10 ml of butanol. Further purification and the fluorometric assay of histamine was performed according to the original method of Shore et al. (1959), which is described briefly under 2.2.4. Recovery of histamine: 65–75%, coefficient of variation 5.2% (n = 10).

2.2.4. Isolation of histamine from tissues and blood by n-butanol extraction (butanol extraction method, Shore et al., 1959)

4.0 ml of the perchloric acids extracts (see 2.2.1.) from tissues, whole blood and plasma were shaken with alkaline butanol. The organic phase was washed with 0.1 N NaOH, saturated with NaCl; histamine was returned to the aqueous phase (0.1 N HCl) with the aid of heptane. After condensation of histamine with o-phthaldialdehyde the fluorescing condensate was stabilized with 3 N HCl instead of 2 M H_3PO_4, as described under 2.2.2. Recovery of histamine: 75–85%, coefficient of variation 4.0 (n = 10).

2.2.5. Isolation of histamine from gastric mucosa, ileum and whole blood of pigs by a modified combined method for chemical and biological identification

Perchloric acid extracts from 20–30 g of gastric mucosa or ileum and 500–700 ml of whole blood of pigs, prepared as described under 2.2.1. were adjusted to pH 6.5 with 2 N NaOH using a glass electrode and distributed to about 50 small columns of Dowex 50 W-X 8 (20 ml of the neutralized extract were applied to each column). After cation exchange chromatography as described under 2.2.2. the undiluted eluates were collected and evaporated to dryness. The dry residue was dissolved in 4.0 ml of twice distilled water, adjusted to pH 11.5–12.0 with 5 N NaOH and shaken for 20 min in a 25 ml glass stoppered Erlenmeyer flask together with 10 ml butanol without the addition of solid NaCl. After washing the organic phase with 5.0 ml of 0.1 N NaOH, saturated with NaCl, histamine returned to the aqueous phase (2.5 ml of 0.1 N HCl) on addition of 15 ml of heptane. This hydrochloric acid solution of histamine was used for the identification of histamine by thin layer chromatography and after neutralization with 0.1 N NaOH for the identification of histamine by enzymatic degradation and by bio-assays.

Recovery of histamine after the preparative procedure: 55–60% (n = 7). The results of histamine assays obtained by the combined method and the modified combined method agreed very well.

2.3. *Identification of histamine*

2.3.1. Identification of histamine according to the criteria of Carlini and Green (1963)

In all tissues of pig and guinea-pig studied, histamine was identified by recording the fluorescence spectrum of the histamine–phthaldialdehyde complex with a Zeiss spectrofluorometer and by measuring the biological activity of the isolated substance on the isolated guinea-pig ileum. The results of the fluorometric assay of histamine were compared with those of the bio-assay. Pharmacological activity of the isolated substance was abolished by the antihistaminic drugs, dimetinden maleate and antazoline, which show a high specifity against histamine in low doses ($0.3 - 2.0 \times 10^{-6}$ M) (Werle and Lorenz, 1970).

2.3.2. Identification of histamine by further chemical and biological methods

For the identification of histamine in gastric mucosa, ileum and whole blood of pig, the following additional tests were carried out:

Thin-layer chromatography on cellulose: After thin-layer chromatography on MN-cellulose (0.25 mm, on glass plates, 20 × 20 cm) in 8 different solvent systems (see table 3), the histamine spots were revealed by spraying the plates with 2 N NaOH followed by o-phthaldialdehyde (1%, in methanol). A bluish fluorescence which appeared within 3–4 min, could be seen after excitation at 366 nm with an UV lamp

(Desaga-Uvis); it was stable for several hours. The high sensitivity of this method allowed the detection of histamine in amounts as low as 20 ng.

The R_f-values of the isolated substance were compared with those of authentic histamine and those of a mixture of these two substances on the same plates.

Degradation by diamine oxidase from pig kidney: Diamine oxidase from pig kidney was purified 150 fold, using a modification of the method of Mondovi et al. (1964). The enzyme activity was determined by measuring the formation of ammonia according to Lorenz et al. (1967) and by the estimation of the formation of Δ-pyrrolidine. Protein was determined by the biuret method and by light absorption at 260/280 nm according to Holmstedt and Tham (1959). 1 kg of frozen pig kidneys was homogenized with two volumes of ice-cold 0.1 M sodium phosphate buffer, pH 7.4 and centrifuged for 30 min at 0°C and 100,000 *g*. The supernatant was heated for 10 min at 60°C, in steel beakers, rapidly cooled in ice cold water with stirring and centrifuged for 15 min at 0°C and 40,000 *g* as described by Dixon and Webb (1964); the precipitate was discarded. The following steps were done at 0–4°C. With the supernatant of the last mentioned centrifugation a fractional precipitation by ammonium sulphate (33 and 60% saturation), was performed, as designed by Mondovi et al. (1964). The second precipitate was dissolved in 50 ml of 0.005 M tris buffer, pH 7.4 and desalted on a column of Sephadex G 25 (4.0 × 60 cm, equilibrated with 0.005 M tris buffer, pH 7.4). The eluate was applied to a column with DEAE cellulose (5.0 × 30 cm, equilibrated with 0.005 M tris buffer, pH 7.4). The effluent was continuously monitored at 280 nm with an Ultraviolet Absorptiometer (LBK 8300 A Uvicord II). The column was washed with about 700 ml of the same buffer and elution was performed with a convex NaCl-gradient, using a constant volume mixing chamber with 200 ml of 0.005 M tris buffer, pH 7.4 and a reservoir with 2 M NaCl, dissolved in the same buffer as that in the mixing chamber. The elution rate was about 120 ml/hr. 8–9 ml fractions were collected and aliquots of the eluate were examined for their enzyme activity. Diamine oxidase was found only in the second protein peak. The fractions of this peak were collected, desalted by applying them to a column of Sephadex G 25 (4 × 50 cm, equilibrated with 0.02 M sodium phosphate buffer, pH 7.4) and lyophilized. This preparation of diamine oxidase was used for the degradation of authentic histamine and the substance isolated from tissues and blood.

The concentrations of the isolated substance in 0.1 N HCl were determined fluorometrically as "histamine equivalents". Authentic histamine was dissolved in equal concentrations in 0.1 N HCl. After neutralizing both solutions with an equal volume of 0.1 N NaOH authentic histamine and the isolated substance were incubated with purified diamine oxidase in centrifuge tubes placed in the shaking incubator (H-350 from Gallenkamp, London), at 25°C under an atmosphere of air. The incubation mixture contained 0.25 ml of the solutions of histamine or the isolated substance, 0.55 ml of 0.2 M sodium phosphate buffer, pH 7.4 and 0.2 ml of diamine oxidase solution (0.1 I.U., dissolved in 0.2 M sodium phosphate buffer, pH 7.4). The reaction was stopped in different incubation samples after 3, 6 and 12 min by the addition of 0.25 ml of 2 N $HClO_4$. After centrifugation for 5 min at 1800 *g*, 1.0 ml of the supernatant was adjusted to pH 6.5 using a glass electrode with 0.1 M sodium phosphate buffer pH 6.5. The residual authentic histamine and the residual "histamine equivalent" were isolated by chromatography on Dowex 50 as described under 2.2.2. and determined fluorometrically. Incubation samples, prepared as described above, but containing the perchloric acid inactivated enzyme were used as blanks.

Identification by bio-assays: After isolation by the modified combined method, histamine concentrations obtained by the fluorometric assay were compared with those obtained by the bio-assay on the isolated guinea-pig ileum and on the blood pressure of the anesthetized cat. The effects of the histamine antagonists, dimetinden maleate and antazoline on the responses of the guinea-pig ileum, described under 2.3.1., and the cat blood pressure (fig. 2) to authentic histamine and to the isolated substance were determined.

2.4. *Histamine release by compound 48/80 and polymyxin B*

The question whether histamine in the pig is released by compound 48/80 and polymyxin B, was studied in 14 young pigs (3–4 months old, 8 female, 3 male and 3 castrated), according to Lorenz et al. (1968).

After 36 hr fasting (Booth, 1969) the animals were anesthetized with pentobarbital (20–40 mg/kg, dose dependent on action) applied intravenously into an ear vein. Following tracheotomy an endotracheal tube was fixed in the trachea, and the animals received artifical respiration. In order to stabilize the circulation during the surgical procedure, 0.5–1.0 litre of Dextran-60 were infused during the surgical treatment described below through a polyethylene catheter in the femoral vein. Dextran showed no hypotensive effects in pigs. The infusion was stopped 15 min before the injection of compound 48/80. The peripheral arterial blood pressure was measured in the common carotid artery by a Ludwig mercury manometer and recorded on a kymograph.

On the right side of the body, the submaxillary and parotid glands, the sternothyroid muscle, and a piece of thymus were removed. After laparotomy a 1–2 g tissue piece was withdrawn from the liver and the spleen. Bleeding was avoided by clamps left within the peritoneal cavity. At the greater curvature of the stomach in the region of fundus, corpus and antrum, little pouches were separated from the residual stomach by bent clamps and removed (see fig. 1).

The defect in the gastric wall was closed by a simple over-and-over suture. The gastric mucosa was stripped from the removed tissue pieces, weighed and, like the other tissue pieces, frozen by liquid nitrogen. The wounds on neck, thorax and abdomen were closed with clamps.

Fifteen minutes after this operation compound 48/80, 3.0 mg/kg or polymixin B, 5.0 mg/kg (both substances dissolved in 0.9% NaCl solution) were applied intravenously through the catheter into the femoral vein. The detergent cremophor-El *, a derivate of ricinoleic acid, which is a very effective histamine liberator in dogs and cats (Lorenz et al., 1970d), was dissolved in 6 volumes of 0.9% NaCl solution and applied in doses of 0.2–2.0 ml/kg in the same way as compound 48/80.

After an initial increase, the blood pressure decreased rapidly and thence more slowly as shown in fig. 3. Ten minutes after the change from the rapid to the slow decrease of blood pressure ("maximal initial decrease of blood pressure"), the animal was sacrificed by bleeding through the catheter in the femoral vein and carotid artery. The tissues corresponding to those which had been removed before administration of the histamine releasers, and the gastric tissues according to fig. 1 (the annular and quadratic pieces) were withdrawn immediately, weighed and frozen by liquid nitrogen. Their histamine content was determined by the Dowex 50 method as described above.

Before and after the operation which preceded the

* We thank very much Bayer, Leverkussen for the gift of cremophor-El.

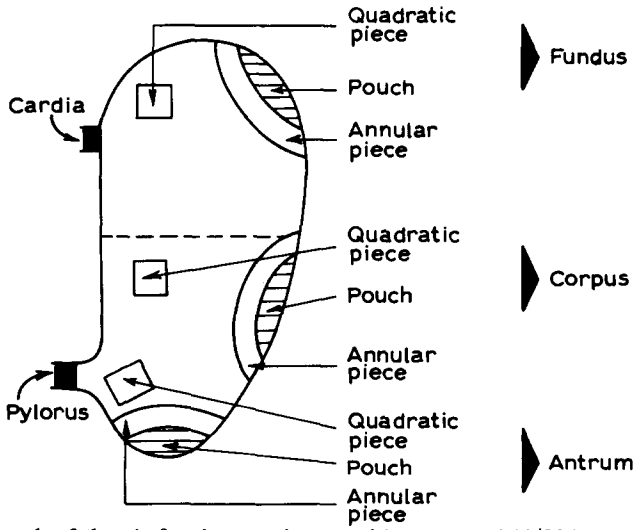

Fig. 1. Preparation of the stomach of the pig for the experiments with compound 48/80 in vivo. The three gastric pouches were removed before the injection of 48/80, the annular and quadratic pieces of the stomach wall about 15 min after 48/80. The corpus pouch was situated always below the middle of the stomach (for anatomy see Ellenberger and Baum, 1912). For further conditions see Methods.

injection of the histamine releasers, 5.0 and 19.5 ml of blood were withdrawn i.v. for histamine determination in whole blood and plasma. After administration of the histamine releasers, these volumes of blood were again withdrawn at the time of the maximal initial decrease of the blood pressure (see fig. 3) and at 2, 5 and 10 min later and used for the determination of histamine in the whole blood and plasma.

2.5. *Studies on the pharmacological actions of histamine and other vasoactive substances in the pig*

The actions of histamine, Betazole, a histamine analogue, and serotonin as well as those of bradykinin and kallidin * on the peripheral arterial blood pressure were studied in anesthetized young pigs (10–15 kg) as described under 2.4. The substances were applied intravenously into the femoral vein.

The stimulation of the gastric and pancreatic secretion by histamine was studied in anesthetized young pigs (20–25 kg). A fistula of the whole innervated stomach was made. A polyethylene tube (diameter 1.2 cm) perforated many times was applied through the duodenum into the stomach according to Lambert et al. (1970). Gastric secretion was stimulated by injection of histamine dissolved in physiological saline into the femoral vein. The gastric juice was collected in 10 min portions, its volume measured and expressed as ml/30 min.

For the studies of pancreatic secretion a polyethylene catheter (diameter 2 mm) was introduced into the major pancreatic duct. The pylorus and the common duct were ligated and the bile was collected through a polyethylene catheter in the hepatic duct. The pancreatic juice was continuously collected, its volume determined and expressed as ml/20 min.

2.6. *Statistical calculations and definitions*

The statistical significance of the results was calculated by the Student's t test for unpaired data and in several cases also for paired data (Snedecor and Cochran, 1967). An Olivetti electronic computer programma 101 was used for these calculations. All histamine values are expressed as histamine dihydrochloride.

* We thank very much Sandoz, Basle for the gift of bradykinin and kallidin.

3. RESULTS

3.1. *Determination of histamine in organs of pig and guinea-pig and in the pig blood*

With pig tissues, the results of the fluorometric assay of histamine after isolation of this amine by the butanol extraction procedure designed by Shore et al. (1959) was compared with the results obtained after isolation of histamine by cation-exchange chromatography on Dowex 50 and those obtained with the combination of these two isolation procedures (table 1). The histamine concentrations obtained after the three purification methods agreed within the limits of experimental error in these three methods.

Since in guinea-pig tissues Michaelson and Coffman (1967) obtained higher histamine values after butanol extraction than after chromatography on an ion-exchanger which separated histamine from spermidine (table 2), the problem was re-investigated using guinea-pigs.

We obtained identical results using the fluorometric assay of histamine after the three purification procedures ($p < 0.5$).

However, in whole blood of pigs we obtained results different to those in tissues. In 16 animals a histamine concentration of 2.57 ± 1.44 μg/ml was obtained after isolation of histamine by butanol extraction and a concentration of 2.13 ± 1.04 μg/ml after the combined method. This difference, about 20%, was statistically significant in the t test for paired data ($p < 0.005$). A comparison of the results after chromatography on Dowex 50 with those after the combined method showed that the values were about 40% higher after the ion-exchange method than after the combined procedure. Since after the last mentioned isolation method only one fluorophore forming substance, which was identical with authentic histamine (see under 3.2.), was present in the solution only the combined method seemed to be suitable for specific determination of histamine in the whole blood of pigs.

For the assay of histamine in pig plasma, however, all three purification procedures were satisfactory. The results after chromatography on Dowex, butanol extraction and the combined procedure agreed very well (0.039 ± 0.016; 0.039 ± 0.013; 0.041 ± 0.022 μg/ml, $n = 5$).

Therefore, in the following experiments histamine

Table 1
Comparison of the histamine concentrations in tissue of young pigs determined fluorometrically after purification of the amine by three isolation procedures.

Tissue	Histamine concentrations (μg/g of weight)		
	Butanol extraction	Chromatography on Dowex 50	Combined method
Tongue	6.2 ± 2.7	8.7 ± 2.3	8.5 ± 2.4
Gastric fundus	38.1 ± 9.8	40.0 ± 3.0	37.0 ± 5.8
Gastric corpus	45.5 ± 31.8	47.1 ± 27.4	49.4 ± 20.0
Gastric antrum	43.2 ± 27.3	43.2 ± 28.7	42.4 ± 27.1
Duodenum	53.6 ± 31.0	54.4 ± 31.3	56.5 ± 29.7
Jejunum	32.2 ± 14.5	33.1 ± 12.5	33.3 ± 13.0
Ileum	31.0 ± 15.8	29.2 ± 14.0	32.0 ± 12.5
Rectum	15.4 ± 0.8	15.9 ± 0.8	16.5 ± 2.6
Liver	5.1 ± 0.4	5.8 ± 0.9	5.7 ± 0.4
Pancreas	2.1 ± 1.1	2.4 ± 1.1	2.1 ± 1.2
Spleen	13.8 ± 2.5	15.9 ± 1.3	15.0 ± 3.7
Kidney	1.3 ± 0.7	1.4 ± 0.4	1.4 ± 0.8

Mean values ± S.D. from three animals. For further conditions see Methods.

Table 2
Comparison of the histamine concentrations in tissues of guinea-pigs determined fluorometrically after purification of the amine by three isolation procedures.

Tissue	Histamine concentrations (μg/g fresh weight)		
	Butanol extraction	Chromatography on Dowex 50	Combined method
Lung	46.5 ± 36.3	42.5 ± 30.5	44.9 ± 25.9
Liver	6.8 ± 1.2	8.0 ± 4.0	6.1 ± 2.4
Heart	12.3 ± 2.8	13.9 ± 4.7	10.8 ± 2.3
Spleen	7.2 ± 0.5	8.5 ± 0.5	9.5 ± 0.9
Kidney	6.3 ± 1.3	7.2 ± 2.3	6.5 ± 1.0

Mean values ± S.D. from six animals. For further conditions see Methods. Between all of the groups no statistically significant differences could be demonstrated ($p < 0.5$)

was determined in pig tissues and plasma by the Dowex 50 method, and in whole blood by the combined method.

3.2. *Identification of histamine in pig and guinea-pig tissues and in the whole blood of pigs*

In all tissues of pigs and guinea-pigs studied and in the whole blood of pigs, histamine was identified by the fluorescence spectrum of the histamine–phthaldialdehyde complex which was identical with that shown by Shore et al. (1959) and by its biological activity on the isolated guinea-pig ileum. The results of the fluorometric assay of histamine agreed with those of the biological assay within the limits of ± 5%. The contraction of the ileum attributed to histamine was completely abolished by dimetinden maleate (0.1 μg/ml bath solution) and antazoline (0.5 μg/ml bath solution). Since two different and relatively specific antihistaminics, in low doses, which are highly specific for histamine (Werle and Lorenz, 1970), inhibited the biological activity of the isolated substances, they were identified as histamine, according to the criteria of Carlini and Green (1963).

However, we tried to confirm the reliability of these criteria by other methods of identification. Histamine preparations from small intestine, gastric mucosa (corpus) and whole blood of pigs (isolated by the modified combined method) were used for thin-layer chromatography on cellulose. The isolated substances, authentic histamine and a mixture of both were revealed by spraying the plates with o-phthaldialdehyde and were shown to have identical R_f values (for small intestinum and whole blood see table 3). The R_f values decreased in acid solvents and increased in more alkaline solvents. Using the Padridge mixture (butanol–acetic acid–water, 50:12.5:62.5) (Stahl, 1967) two R_f values (0.18 and 0.30) were obtained with authentic histamine and the isolated substances which are typical for free amines in acid solvents (Stahl, 1967). Therefore, histamine and the isolated substances were identical by means of thin-layer chromatography in all solvents studied. No further fluorophore-forming substance could be shown on the plates after spraying them with o-phthaldialdehyde. The specificity of the fluorometric assay of histamine was further examined by the degradation of the three isolated substances and of authentic histamine with purified diamine oxidase from pig kidney. The velocity of this degradation was the same for authentic histamine as for the isolated substance (for an example see table 4). These results indicate the identity of the isolated substances with authentic histamine.

Finally, we studied the biological activity of the three isolated substances on a second biological test system, the blood pressure of the anesthetized cat. As shown for the substance isolated from pig whole blood (fig. 2), they had the same pharmacological properties as authentic histamine. The whole biological activity was specifically abolished by dimetinden maleate. Therefore, authentic histamine and the substances isolated from the small intestinum, gastric mucosa and whole blood were considered to be identical.

Table 3
Identification of the substance isolated from the small intestinum and whole blood of the pig as histamine by its R_f values after thin-layer chromatography on cellulose MN 300.

No.	Solvents (v/v)	R_f values					
		Histamine		Isolated substance		Histamine + isolated substance	
		Intestinum	Blood	Intestinum	Blood	Intestinum	Blood
1	Ethanol–diethylether–ammonia (25%)–water (48:60:6:12)	0.50	–	0.48	–	0.49	–
2	Ethanol–ammonia (25%) –water (100:10:10)	0.54	0.56	0.54	0.59	0.54	0.59
3	Ethanol–ammonia (25%) (80:20)	0.68	0.67	0.68	0.68	0.68	0.68
4	Methanol–acetic acid (125:20)	0.13	0.12	0.12	0.14	0.12	0.14
5	Propanol–ammonia (25%) (80:20)	–	0.56	–	0.56	–	0.56
6	Butanol–pyridine–water (40:40:40)	0.34	0.36	0.34	0.35	0.32	0.35
7	Butanol, saturated with N HCl	0.05	0.05	0.05	0.07	0.05	0.07
8	Phenol (100 g)–water (30 ml)	0.29	–	0.27	–	0.29	–

Mean values from 3–8 determinations. For further conditions see Methods.

Table 4
Comparison of the reaction velocities for the degradation of authentic histamine and the substance isolated from the pig's whole blood by diamine oxidase.

Incubation time (min)	Residual histamine after incubation with diamine oxidase (nmoles/incubation sample)	
	Isolated substance	Authentic histamine
0	5.1	5.0
3	3.6	3.4
6	1.8	1.9
12	1.3	1.5

Mean values from two determinations. Purification of the enzyme and incubation conditions see Methods.

3.3. *Distribution of histamine in tissues and blood of adult and young pigs*

The histamine concentrations of several tissues in the adult pig are amongst the highest of all normal mammalian tissues (table 5). Especially rich in histamine were stomach, duodenum, small intestinum and lung. The lowest histamine contents were found in salivary glands and pancreas (< 10 μg/g). In the alimentary canal, the histamine concentrations increased from the oesophagus up to the jejunum and decreased from there down to the rectum. It reached its highest values at the beginning of the jejunum, decreased by 21% down to the end of the jejunum and increased a second time at the beginning of the ileum. From there the histamine content decreased continuously down to the end of the ileum. The terminal ileum showed only 55% of the histamine concentration found in the proximal jejunum (table 6).

In 3 month old pigs, the histamine concentrations of nearly all organs were lower than in those of adult animals (table 7). However, the relationship between the histamine contents of the tissues in young and adult pig were not the same in all tissues. In tongue, stomach, duodenum, salivary glands and pancreas, this relationship was 2:1 (adult : young), in the liver 3:1, in several parts of the small intestinum 4:1, in the lung 6:1 and in the gall bladder 7:1.

The histamine concentration of abdominal skin, tonsils, thyroid gland and atria of the heart were relatively high (table 7). The thyroid glands of young pigs had similar histamine contents to those of adult animals (Werle and Lorenz, 1966).

The histamine concentration of whole blood in young pigs was 2.1 ± 0.8 μg/ml (1.3–4.2 μg/ml, n = 12). In pig plasma, the histamine concentration was 0.05 ± 0.04 μg/ml (0.025–0.13 μg/ml, n = 7). The distribution of histamine between corpuscular elements and plasma in the blood of pigs indicated that about 98% of histamine in the whole blood was localized in cells.

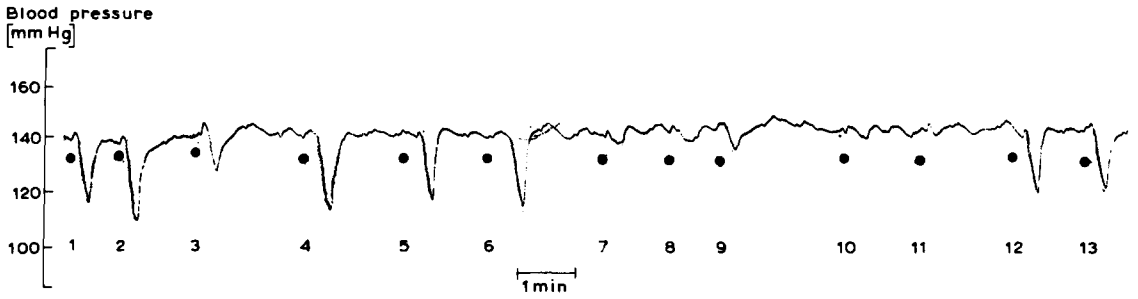

Fig. 2. Identification of the substance isolated from the whole blood of pigs as histamine by bio-assay on the blood pressure of the anesthetized cat. Female cat, 3 kg, sodium pentobarbital (15 mg/kg), tracheotomy and spontaneous respiration, measurement of the blood pressure in the common carotid artery with a Ludwig mercury manometer. i.v. injection into the femoral vein: (1) 0.1 μg acetylcholine; (2) 0.2 μg acetylcholine; (3) 2 μg bradykinin; (4) 4 μg bradykinin; (5) 0.5 μg histamine; (6) 0.5 ml pig's whole blood preparation (see Methods); (7–9) 1 mg dimetinden maleate; (10) 1μg histamine; (11) 1 ml of pig's whole blood preparation; (12) 0.2 μg acetylcholine; (13) 4 μg bradykinin. 1 ml of pig blood corresponds to 1 μg of histamine determined fluorometrically.

Table 5
Histamine concentrations in the digestive tract and in the lung of adult pigs.

Tissue	Histamine concentration (μg/g fresh weight)	
	Mean ± S.D.	Range
Tongue	14.2 ± 12.2	3.6 – 32.4
Submaxillary gland	4.0 ± 2.3	2.0 – 7.7
Parotid gland	5.5 ± 1.4	3.6 – 7.5
Oesophagus, proximal	36.5 ± 16.5	15.3 – 63.5
distal	33.2 ± 10.7	17.6 – 49.0
Stomach, fundus	150.4 ± 66.7	88.0 – 277.0
corpus	99.7 ± 26.5	61.5 – 134.0
antrum	102.2 ± 48.8	27.2 – 172.0
Duodenum, proximal	130.1 ± 50.5	96.6 – 229.0
middle	147.1 ± 77.4	78.0 – 264.0
distal	143.1 ± 59.5	93.6 – 229.0
Jejunum, proximal	265 ± 15	247 – 282
distal	229 ± 6	225 – 236
Ileum, proximal	237 ± 29	195 – 257
distal	183 ± 20	158 – 208
Colon, proximal	127.2 ± 56.0	90.0 – 224.7
distal	109.3 ± 23.0	79.8 – 144.0
Rectum	73.3 ± 42.5	26.6 – 142.8
Liver	17.6 ± 2.6	14.4 – 21.5
Gallbladder	56.6 ± 9.0	20.2 – 73.8
Pancreas	4.1 ± 0.8	2.5 – 6.2
Lung	309.0 ± 46.3	262 – 370

Mean values from 6 animals. From each pig, all the tissues named in the table were studied. The proximal part of the oesophagus comes from the pharynx up to the bifurcation of trachea, the distal part from this point down to the cardia. The three parts of the duodenum correspond to the three parts of the duodenal C. From the parts of the small intestinum corresponded each to about four m of intestinum. The proximal part of the colon corresponded to the part containing convolutions, the distal to the straight part (see Ellenberger and Baum, 1912). In all experiments, the whole wall of the digestive tract was studied.

Table 6
Histamine concentrations of different parts of the small intestinum in adult pigs.

Part of the small intestinum (length in meters)	Histamine concentration (μg/g fresh weight)	
	Mean ± S.D.	Range
Jejunum		
1st	282 ± 174	55–495
2nd	261 ± 176	43–480
3rd	268 ± 179	46–498
4th	247 ± 170	40–440
5th	236 ± 162	23–384
6th	225 ± 158	11–380
7th	225 ± 160	20–380
Ileum		
1st	254 ± 139	56–412
2nd	257 ± 141	69–408
3rd	243 ± 135	25–385
4th	195 ± 103	29–302
5th	208 ± 126	21–356
6th	184 ± 122	17–307
7th	180 ± 102	43–340
8th	158 ± 98	31–292

Mean values from 6 animals. For the distribution between jejunum and ileum see Ellenberger and Baum (1912). For all experiments the whole intestinum wall was used.

3.4. *Histamine release by compound 48/80, polymyxin B and experiments with the detergent cremophor EL*

To obtain information about the properties of histamine stores in the pig, histamine release from tissues into the blood was studied after injection of typical mastcell-histamine liberators (Lorenz et al., 1968, 1969).

3.4.1. Histamine release into the plasma

Initially, compound 48/80 (MacIntosh and Paton, 1949) was given to young pigs in doses which had been used in dogs (Lorenz et al., 1969) and its action on the peripheral arterial blood pressure was studied (fig. 3). In contrast to observations in other species (Lorenz et al., 1969), the typical decrease in blood pressure, as seen in dogs (Lorenz et al., 1968), was preceded by short-lasting hypertension. The hypertensive phase was independent of sex, weight or the altitude of the arterial pressure before the injection of 48/80 and was observed in seven cases out of nine showing any reactions toward compound 48/80 (table 8). The average increase in blood pressure was 50 mm Hg and was followed by a decrease of 80 mm Hg. A further peculiarity of the pig was the relatively large number of animals resistant to a high dose of 48/80 (25%), a resistance that was not found in dogs.

Further actions of compound 48/80 in the anesthetized pig were observed: Nasal and salivary secretion, hyperventilation, sometimes bronchospasm, clonic and tonic spasms, a relatively strong hyper-

Table 7
Histamine concentrations in the digestive tract and in some other tissues of young pigs.

Tissue	Histamine concentrations (μg/g fresh weight)	
	Mean ± S.D.	Range
Head		
Tongue, tip	7.6 ± 2.7	4.3– 11.2
body	8.5 ± 4.6	3.3– 11.5
root	4.2 ± 1.8	2.2– 11.5
Soft palate	11.0 ± 4.0	7.7– 15.5
Palatine tonsil	21.1 ± 4.5	16.2– 26.8
Pharynx	5.4 ± 2.6	3.1– 8.5
Submaxillary gland	2.0 ± 0.8	1.1– 4.0
Parotid gland	1.8 ± 1.1	0.7– 4.4
Sublingual gland	3.2 ± 2.7	1.3– 7.2
Neck and thorax		
Oesophagus, proximal	10.8 ± 1.2	5.1– 12.4
distal	12.1 ± 2.6	7.0– 14.7
Thyroid gland	13.5 ± 8.1	5.2– 27.2
Thymus	9.7 ± 5.5	3.0– 20.0
Lymph node	10.5 ± 5.8	1.3– 15.4
Sternothyroid muscle	0.9 ± 0.1	0.7– 1.1
Lung	54.0 ± 18.1	26.6– 71.0
Heart, atria	12.2 ± 4.1	4.8– 18.0
right ventricle	6.2 ± 3.1	2.0– 12.0
left ventricle	5.0 ± 3.8	1.9– 9.2
Diaphragm	6.5 ± 4.8	1.9– 11.5
Abdomen		
Stomach, fundus	44.5 ± 19.4	14.0– 81.0
corpus	55.1 ± 39.6	21.0–147.0
antrum	30.3 ± 18.1	11.3– 63.5
Duodenum	70.0 ± 36.5	26.6–159.0
Jejunum, proximal	63.3 ± 32.9	22.9–110.4
distal	47.5 ± 28.9	14.8– 98.0
Ileum, proximal	60.1 ± 32.8	23.5– 98.0
distal	45.9 ± 17.1	30.0– 71.0
Caecum	29.2 ± 4.0	23.2– 31.6
Colon, proximal	26.8 ± 7.4	11.7– 33.0
distal	24.7 ± 1.0	23.0– 26.8
Rectum	16.7 ± 3.9	13.2– 23.3
Liver	7.8 ± 4.3	2.3– 17.5
Gallbladder	7.7 ± 5.3	2.7– 13.3
Pancreas	2.6 ± 1.6	1.1– 6.0
Spleen	17.3 ± 6.2	8.8– 33.2
Abdominal skin	16.2 ± 1.6	15.0– 18.0
Kidney	1.1 ± 0.4	0.7– 1.8
Suprarenal	1.6 ± 0.6	0.9– 2.4

Mean values from 14 animals. For definitions of the different intestinal parts see table 1. The abdominal skin was removed around the navel. The whole wall of the gut was used.

aemia of the skin with cyanosis and petechiae, formation of wheals, especially on the ears and protrusion of the anus. After laparotomy a strong hyperaemia of the intestinum was observed. The intestinal mucosa showed a spotty redness. Small haemorrhages were seen in the gastric mucosa and, despite fasting, the stomach was full of a weak acid secretion, containing bile.

All of these actions – with the exception of the initial increase of blood pressure, which is a peculiarity of pigs – could be explained by known actions of released histamine (Rocha e Silva, 1966a). Therefore, an attempt was made to demonstrate release of histamine by 48/80 in pigs by determination of the amine in whole blood and plasma. In whole blood, no significant increase of the histamine concentration was found after injection of 48/80, but rather a decrease at the point of time of the maximal initial decrease of the blood pressure (fig. 4a).

However, the histamine concentration in the plasma increased by 160% at the time of the maximal initial decrease of blood pressure and remained elevated until the end of the experiment (fig. 4b). The increase of the histamine concentration in the plasma of pigs agreed quantitatively with the increase of the histamine concentration in whole blood of dogs after application of several histamine releasers (about 0.1 μg/ml blood) (Messmer et al., 1969; 1970). Since the sensitivity of the pig towards histamine (see under 3.5.1.) and the blood pressure response to 48/80 were similar to those of dogs, compound 48/80 apparently released histamine in the pig as in most of the mammals studied. Furthermore, correlation between the decrease in blood pressure and increase of the histamine concentration in the plasma was highly significant (fig. 5).

The tonic-clonic spasms of the animals after compound 48/80 seemed to be of central origin and could not be observed after histamine injection. However, they were seen only in those animals, with a decreased blood pressure and an increased plasma histamine level. Therefore, histamine release in parts of the central nervous system of pigs after 48/80 might be considered.

In two experiments, polymyxin B decreased blood pressure about 70 mm Hg and increased plasma histamine concentration about 120%; the histamine concentration of whole blood remained unchanged.

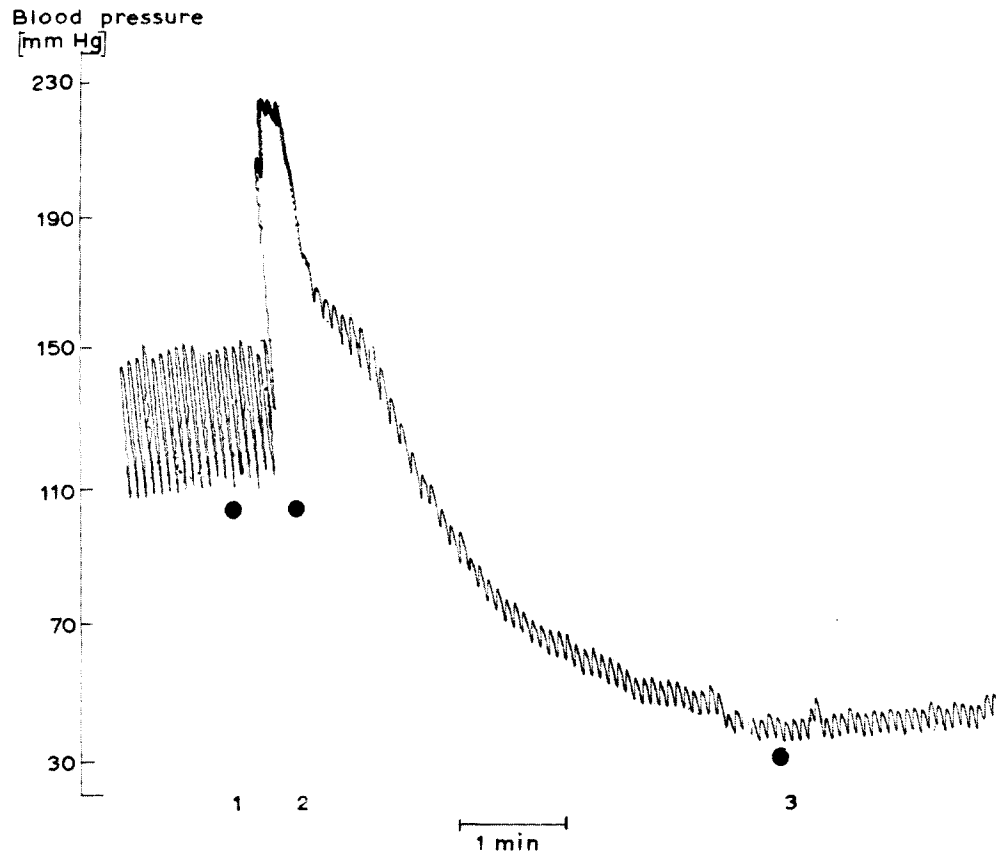

Fig. 3. Action of compound 48/80 on the peripheral arterial blood pressure of the young pig. Female pig, 16 kg, sodium pentobarbital (25 mg/kg), tracheotomy and artificial respiration, measurement of the blood pressure in the common carotid artery by a Ludwig mercury manometer. i.v. injection of compound 48/80 (3 mg/kg) into the femoral vein. (1) Injection of 48/80; (2) maximal initial increase of blood pressure; (3) maximal initial decrease of blood pressure. 10 min after the last point of time, the animal was bled and the tissues removed, as shown in tables 9 and 10.

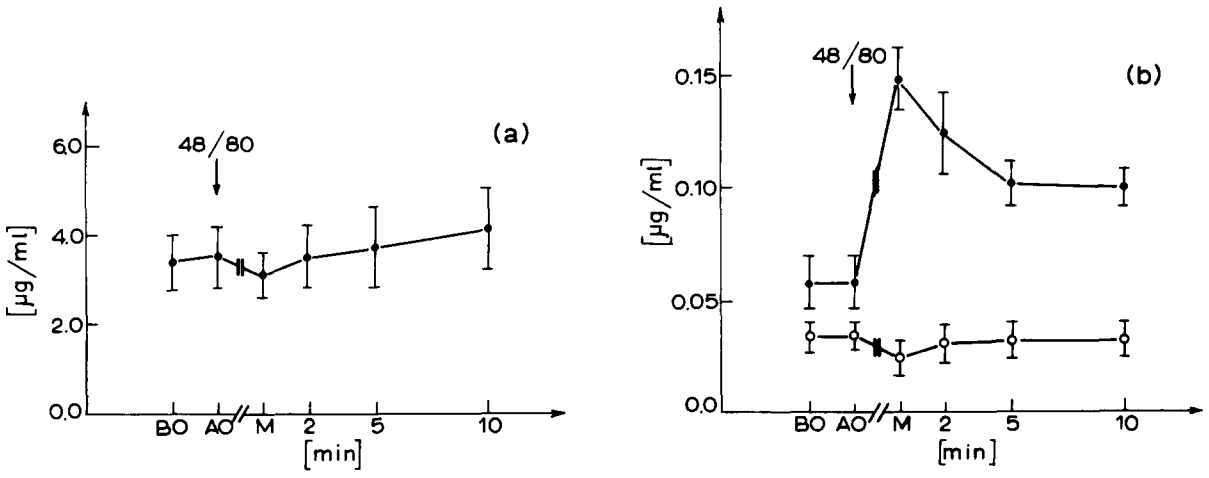

Fig. 4. Histamine concentrations in the whole blood (a) and plasma (b) before and after the injection of compound 48/80 into young pigs. Histamine concentrations in µg/ml blood or plasma as mean ± S.E. B.O. = before operation, A.O. = after operation, M = maximal initial decrease of blood pressure. 2, 5, 10 = 2, 5, 10 min after the maximal initial decrease of blood pressure. (4a) Histamine concentrations in the whole blood, n = 4. No significant changes during the experiments. (4b) Histamine

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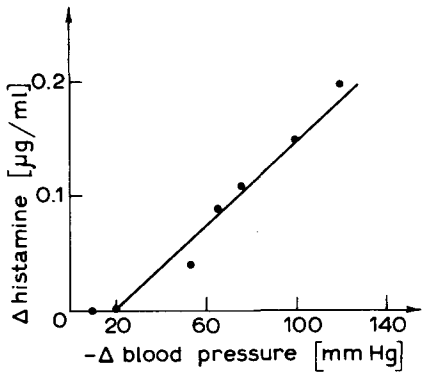

Fig. 5. Correlation between the decrease of the peripheral arterial blood pressure and the increase of the histamine concentrations in the plasma of pigs after injection of 48/80. The decrease of blood pressure means the maximal initial decrease as shown in figs. 3 and 4. $r = 0.98, p < 0.001$. Increase of histamine (Δ histamine) in µg/ml plasma, decrease of blood pressure in the common carotid artery (−Δ blood pressure) in mm Hg.

Since detergents release histamine in the dog (Halpern, 1956), cremophor El, a very effective histamine liberator in dogs and cats (Lorenz et al., 1970d), was given to pigs. However in pigs, doses ten times higher than those used in dogs (2.0 mg/kg), did not produce hypotension greater than that produced by the same volume of 0.9% NaCl; plasma histamine concentration was not increased. Therefore, since the pig was resistant to the histamine releasing activity of the detergent cremophor El, these detergents may only release histamine in carnivora.

3.4.2. Histamine release from tissues

After injection of compound 48/80, histamine concentrations of whole blood, plasma and of several tissues were studied. To standardize the experimental conditions in each of the animals, the tissues were withdrawn 10 min after the maximal initial decrease of blood pressure (fig. 3), that is about 15 min after the injection of 48/80 (table 8). Animals which showed hypotensive reactions of more than 30 mm Hg and a significant increase of the plasma histamine concentration, were compared with those which showed none of these actions of 48/80 and those which were only sham-operated and sham-injected (the same volume of 0.9% NaCl was injected as in the pigs treated by 48/80, and the tissues were withdrawn 15 min after the injection of the saline solution).

The two last mentioned groups, i.e. those receiving 48/80 and showing no response and those receiving saline, showed no significant changes in the histamine content of their tissues (table 10). However, in those animals which developed hypotension and in which plasma histamine concentration was increased after treatment with 48/80, the histamine concentrations decreased in some of the tissues studied, increased in others and remained unaltered in others (table 9).

In the liver, the histamine content decreased by 24%. This release was smaller than in the liver of dogs after injection of histamine liberators (Ojers et al., 1941; Copenhaver et al., 1953), where about 40% or more of the histamine disappeared from the tissue. From the submaxillary and parotid glands up to 50% of the histamine was released (significant in the t test for paired data). This finding is in contrast to results in adult dogs (Lorenz et al., 1968) and rats (Brodie et al., 1966), where the submaxillary gland stored histamine in short-lasting experiments. Compound 48/80 released 40% of the histamine from the sternothyroid muscle (table 9).

However, in gastric mucosa, the histamine concentration increased after injection of compound 48/80. Similar results were obtained in dog (Lorenz et al., 1969), cat and rat (Beaven et al., 1967). Since the increase of the histamine content in the gastric mucosa of these species was probably due to enhanced uptake of histamine, this mechanism is also assumed to occur in pigs.

Histamine concentrations of spleen and thymus were unchanged after injection of 48/80 (table 9).

To determine where tissue pieces should be removed for determination of the histamine

Table 8
Actions of compound 48/80 on the peripheral arterial blood pressure of young pigs.

Pig. No.	Sex	Weight (kg)	Initial blood pressure (mm Hg)	Action of 48/80 on blood pressure		Time of maximal initial decrease after 48/80 injection (min)
				Initial maximal increase (mm Hg)	Initial maximal decrease following the increase (mm Hg)	
1	c	16	100	0	0*	–
2	f	15	130	0	0*	–
3	c	13	140	38	120	2
4	f	17	125	0	40	3
5	m	14	120	76	70	3
6	f	14	150	62	95	4
7	f	18	140	20	100	4
8	c	15	150	72	66	6
9	m	20	150	30	70	5
10	m	15	120	0	0*	–
11	f	12	120	0	90	5
12	f	16	150	70	118	5
Mean Values ± S.D.	–	15.4 ± 2.3	133 ± 16	52 ±** 23	82 ±** 29	4.3 ±** 1.3

Sex: c = castrated, f = female, m = male.
For definitions of the blood pressure phases see fig. 3.
For the whole procedure see Methods and fig. 3.

* Compound 48/80 was without effect.
** The animals resistant to 48/80 were excluded from the calculations.

concentration in the gastric mucosa after injection of compound 48/80, the following experiments were made: Two tissue pieces were removed from the areas of the stomach containing the gastric pouches before the injection of 48/80: one annular piece around the defects of the stomach wall due to the remotion of the pouches, and one quadratic piece remote from the defect as seen in fig. 1.

Annular and quadratic pieces from the corpus and antrum showed similar increases in histamine concentration after 48/80. In the fundus however, the increase was significantly lower in the annular piece than in the quadratic piece; in five experiments the annular tissue piece contained 26.8 ± 8.6 μg/g, the quadratic piece 47.6 ± 11.3 μg/g ($p < 0.05$). In seven experiments with corpus and antrum, the corresponding values were 46.7 ± 12.8 μg/g and 43.8 ± 14.1 μg/g ($p < 0.7$) respectively 28.8 ± 14.7 μg/g and 27.5 ± 8.8 μg/g. Disturbances of the microcirculation may be responsible for the different histamine concentrations of the annular and quadratic fundic tissue pieces. Therefore, for experiments with histamine releasers, tissue pieces should be removed from the same area of the gastric mucosa before and after the injection of the drug (Lorenz et al., 1969), and, not from adjacent areas, because the mucosa may have been crushed by the surgical treatment.

3.5. *Pharmacological actions of histamine in the pig*

3.5.1. Actions on the peripheral arterial blood pressure

Histamine decreased peripheral arterial blood pressure in the pig (fig. 6a) as already shown in most of the other mammalian species (for a survey see Rocha

Table 9
Histamine concentrations of some tissues of the young pig before and after the injection of 48/80.

Tissue	Histamine concentration (μg/g) Before 48/80	After 48/80	Change in ± %	p Value
Liver	7.8 ± 1.5	5.9 ± 1.4	− 24	< 0.05
Gastric mucosa,				
fundus	27.3 ± 10.4	38.8 ± 5.0	+ 42	< 0.05
corpus	32.6 ± 12.5	48.1 ± 11.4	+ 48	< 0.05
antrum	15.9 ± 10.2	37.1 ± 13.1	+ 131	< 0.02
Submaxillary gland	2.12 ± 0.41	1.27 ± 0.65	− 40	< 0.05
Parotid gland	1.75 ± 0.47	0.88 ± 0.67	− 50	< 0.05
Thymus	5.5 ± 2.4	5.7 ± 2.5	—	—
Spleen	13.6 ± 4.9	13.6 ± 4.2	—	—
Sternothyroid muscle	1.06 ± 0.26	0.65 ± 0.06	− 39	< 0.02

Mean values from 6 animals. In the stomach the mucosa was removed from the musculature by separating it in the submucosal layer. The decrease of blood pressure after the injection of 48/80 was at least 40 mm Hg in every animal studied. Before 48/80 right submaxillary gland, parotid gland and sternothyroid muscle were removed, after 48/80 the left ones. For further conditions see Methods.

Table 10
Histamine concentrations of some tissues of the young pig before and after injection of compound 48/80 in animals without hypotensive reactions and in sham-operated animals.

Tissue	Histamine concentrations (μg/g)			
	Animals resistant to 48/80		Sham-operated animals	
	Before 48/80	After 48/80	Before NaCl	After NaCl
Liver	6.4	7.1	5.2	5.9
Gastric mucosa				
fundus	35.2	32.2	19.3	20.3
corpus	47.0	43.0	28.5	26.3
antrum	39.5	37.0	20.9	21.8
Submaxillary gland	2.2	2.3	1.7	1.6
Parotid gland	2.9	2.8	1.6	1.5
Thymus	8.9	7.1	3.7	3.5
Spleen	17.4	17.2	7.2	8.6
Sternothyroid muscle	0.9	0.9	0.4	0.3

Mean values from 3 animals, which were sham-operated and 3 animals which were resistant to 48/80 (no hypotensive reaction, no increase in plasma histamine levels, but a normal sensitivity to exogenous histamine with respect to blood pressure response). For further conditions see Methods and table 9.

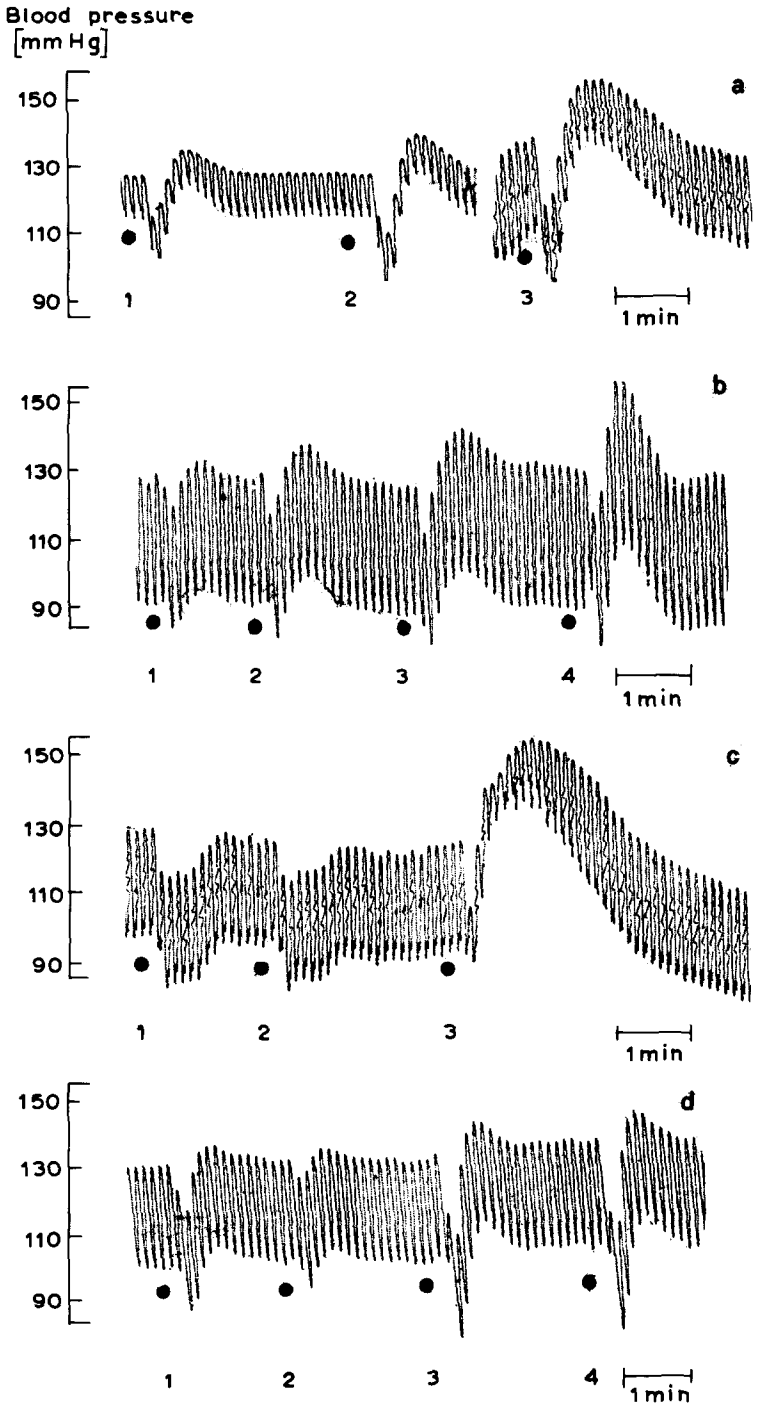

Fig. 6. Actions of histamine, betazole, serotonin and kinins on the peripheral arterial blood pressure of the young pig. Male pig 14 kg, sodium pentobarbital (35 mg/kg), tracheotomy, artificial respiration. Measurement of the blood pressure by a Ludwig mercury manometer, i.v. injection into the femoral vein: (a) histamine (μg): (1) 50, (2) 100, (3) 1000; (b) betazole (mg): (1) 2.5, (2) 5, (3) 10, (4) 20; (c) serotonin (μg): (1) 50, (2) 100, (3) 1000; (d) kinins (μg): (1) 1.0 kallidin, (2) 1.0 bradykinin; (3) 2.0 kallidin, (4) 2.0 bradykinin.

e Silva, 1966). The pig, however, was relatively insensitive toward histamine. The doses necessary for evoking a significant blood pressure reaction were similar to those required in the dog, and sometimes even higher.

Unexpectedly, histamine caused a biphasic blood pressure response which was more pronounced after the injection of higher doses (fig. 6a). It consisted of an initial decrease followed by an increase of blood pressure. Betazole, a histamine analogue used for secretory studies in the digestive tract (Lorenz et al., 1970b), also produced a biphasic blood pressure response whereas higher doses, used for clinical purposes, often only increased blood pressure (fig. 6b), a finding contrary to those in dogs and cats (Lorenz et al. 1970b).

The effect of higher doses of histamine on the blood pressure probably explains the initial hypertension in the experiments with compound 48/80. It seems possible that in the pig, histamine releases relatively high amounts of catecholamines, a mechanism which occurs with much higher doses in other species (for a survey see Holtz and Palm, 1966). Also serotonin produced a biphasic blood pressure curve (fig. 6c) similar to that observed in dogs (Lorentz et al., 1970b). Bradykinin and kallidin, however, in doses equieffective to those of the biogenic amines used in this study, decreased blood pressure without producing a significant hypertensive phase (fig. 6d).

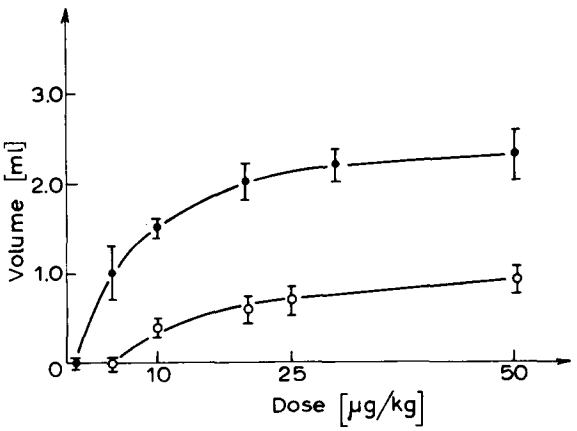

Fig. 7. Stimulation of gastric and pancreatic secretion in young pigs by histamine. Dose-response curves from 4–5 pigs. Acute fistula of the whole innervated stomach and the pancreas as described under Methods. ●——● gastric secretion (injection of histamine into the femoral vein). ○——○ pancreatic secretion (injection of histamine into the pancreatoduodenal artery). Mean values ± S.E., volume of gastric secretion in ml/30 min, volume of pancreatic secretion in ml/20 min. The basal secretion of both glands was subtracted.

3.5.2. Stimulation of the secretion of exocrine glands in the digestive tract

Histamine stimulated salivary, gastric and pancreatic secretion in pigs as in other mammalian species (Lorenz et al., 1968a; Lorenz and Pfleger, 1968). Dose-response curves are presented for gastric and pancreatic secretion (fig. 7). It can be seen that the secretory response from the acute fistula of the whole innervated stomach was much lower in pigs than in dogs (Feifel et al., 1970), whereas the secretion rates from the acute fistula of the pancreas corresponded to those obtained in the dog (Lorenz et al., 1968a).

4. DISCUSSION

In the last few years, the pig has been shown to be an excellent laboratory animal for many studies in the fields of biology and medicine (Bustad and McClellan, 1966). For elucidation of the physiological and pathological functions of histamine in mammals, this species seems to be very suitable for many reasons, some of which are:

1) Since histamine concentrations are relatively high in most pig tissues, there are no essential problems for its determination.

2) The pig is sensitive toward classical histamine releasers, such as compound 48/80 and polymyxin B. Detergents, however, are ineffective. Therefore, the pig resembles man more closely than do some other higher mammals e.g. the dog and the cat (Lorenz et al., 1969).

3) The enzymes which form and metabolize histamine, are highly active in pig tissues so far as is studied, i.e. the histidine decarboxylase of the gastric mucosa (Lorenz et al., 1969a; 1969b) and thyroid gland (Werle and Lorenz, 1966; Lorenz and Werle, 1967), the diamine oxidase of the kidney (Zeller, 1942; Kapeller-Adler, 1949; Mondovi et al., 1964) and the histamine methyltransferase of liver (Lindahl,

1960) and gastric mucosa (Lorenz et al., 1970. 1970e).

In this study much emphasis was placed on the examination of the specificity of our test and on the identification of histamine. Since Carlini and Green (1963) found that in rat brain some substances not identified by the authors interfered with the fluorometric assay of histamine, the specificity of the method of Shore et al. (1959) was questioned generally by several workers (Michaelson and Coffman, 1967; Aures et al., 1969). Kremzner and Pfeiffer (1966) identified spermidine as the most common interfering substance in the brain which forms a fluorescing condensation product with o-phthaldialdehyde. However, the fluorescence of this fluorophore is 60 times less intense than the histamine fluorophore and it can be separated from histamine by several ion-exchange procedures, but not by butanol extraction according to Shore et al. (1959).

Histamine is separated from spermidine by chromatography on Dowex 50 as shown by Lorenz et al. (1970, 1970c). Therefore, in pig tissues this substance did not interfere with the determination of histamine after all three purification procedures, including butanol extraction according to Shore et al. (1959) because all these procedures gave the same histamine values within the limits of experimental error.

In some guinea pig tissues studied, we obtained the same results as in the pig. Therefore we cannot support the statement of Michaelson and Coffman (1967) that in the guinea-pig tissues (with exception of brain!) the original method of Shore et al. (1959) is generally not suitable for the determination of histamine.

The distribution of histamine in many tissues of the pig was first studied in this paper. However, histamine concentrations of single organs had already been determined before this study with the aid of biological and chemical methods: salivary glands (Werle and Lorenz, 1964; Lorenz et al., 1968), palatine and nasopharyngeal tonsils (Lorenz et al., 1968), stomach (Werle and Zeisberger, 1952; Riley and West, 1956; Lorenz and Pfleger, 1968; Lorenz et al., 1968b; Lorenz et al., 1969), liver (Riley and West, 1953; Graham et al., 1969), gallbladder (Lorenz et al., 1969), pancreas (Lorenz et al., 1969), lung (Riley and West, 1953; Graham et al., 1956), aorta (Riley and West, 1953; Riley, 1959), whole blood (Baxter et al., 1954), spleen (Riley and West, 1953), thymus (Lorenz et al., 1968), abdominal skin (West, 1959), thyroid gland (Werle and Lorenz, 1966; Waite et al., 1967), gastric juice (Thouvenot and Harichaux, 1961). The results obtained in these studies agree with those of this paper.

The histamine concentrations of nearly all organs are lower in the young pig than in adults. This finding, which has been shown in several mammals, as for instance in the skin of rat (West, 1959), the lung of guinea-pig (Trethewie, 1947) and cats (Feldberg and Kellaway, 1937), can be explained by an increase of the mast cell density in relation to age.

The histamine concentrations in pig whole blood and plasma were unexpectedly high. Comparison with those of other mammals (Lorenz and Werle, 1969) shows that the pig is the species with the second highest histamine concentration in the whole blood of all mammals studied. The histamine content of pig plasma is about 100 times higher than that of man (Adam et al., 1957; Graham et al., 1968; Lorenz et al., 1970a, b, c).

Some of the diseases of the pig take their course under the clinical picture of an allergosis, especially infective gastro-intestinal disorders (Moon et al., 1970) or diseases of the skin. The high histamine concentrations of these tissues could be determining factors of the clinical picture.

The success of homologous liver transplantation in pigs might be due not only to the pecularities of liver circulation (Messmer, 1970) but also to the fact, that histamine is released to a smaller extent from pig liver than from dog liver by typical releasers of mast cell histamine. Histamine release from typical mast cells in the course of immunological processes, hypoxia and hypothermia, could determine together with other factors, the clinical picture of acute or peracute graft rejection.

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