Histamine-Containing Peripheral Neuronal and Endocrine Systems

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An immunohistochemical method was developed to detect histamine in tissues. The aim of this study was to reveal the cellular stores of histamine in the gastrointestinal tract, pituitary, and adrenal gland. Histamine-containing nerve fibers were found in both rat and guinea pig gut. The origin of at least some of these fibers in the rat ileum was the submucous ganglion cell layer. In the rat stomach, numerous enterochromaffin-like cells exhibited histamine immunofluorescence, and endocrine cells in the ileum and jejunum contained histamine. Only mast cells contained histamine in the neurohypophysis. A large number of process-bearing cells in the guinea pig but not in the rat adrenal medulla contained histamine.

The study shows that histamine is present in peripheral nerves and endocrine cells in addition to mast cells, and may function as a neurotransmitter or hormone.

KEY WORDS: Histamine; Immunohistochemistry; Enterochromaffin cells; Gut nerves; Pituitary gland; Adrenal medulla

Introduction

There is considerable evidence that histamine functions as a neurotransmitter in the central nervous system (Green et al., 1978; Schwartz et al. 1980). In fact, histamine-immunoreactive neurons have been demonstrated in the rat brain using antibodies against histamine itself (Wilcox and Seybold, 1982; Panula et al., 1984b). Much less is known about the peripheral neuronal system which might use histamine as a transmitter, because there has been no specific way to detect histamine-containing neurons in the peripheral tissues.

Histamine stimulates sympathetic ganglia (Trendelenburg, 1954; Iorio and Mclsaac, 1966; Brezenoff and Gertner, 1972). It also depolarizes adrenal chromaffin cells and releases catecholamines from chromaffin cells (Douglas et al., 1967; Staszewska-Barczak and Vane, 1965). Histamine is involved in the regulation of normal gastrosecretory function (Code, 1965; Kahlson and Rosengren, 1968), and the amine has been demonstrated in the enterochromaffin-like cells in the stomach using the α-phthalaldehyde fluorescence method (Häkanson and Owman, 1967; Ehinger et al., 1968). However, this method may have low sensitivity, because it failed to reveal the cellular stores of histamine in the nervous tissue. Histamine also appears to excite cholinergic ganglion cells in the gastric wall presynaptically and the smooth muscle of the gastric wall directly (Paton and Vane, 1963). Actions of histamine on intestinal smooth muscle vary with species and region (Douglas, 1980), but the general effect is contraction. However, little is known about the source of histamine in the intestine. Systemic administration of histamine stimulates ACTH secretion (Cowan, 1975; Dallman and Yates, 1968) by an indirect mechanism (Ganong, 1963), and histamine may regulate LH secretion (Weiner and Ganong, 1978). There is also some evidence that histamine may be involved in the physiological regulation of prolactin secretion, probably by an indirect mechanism (Weiner and Ganong, 1978; Liberton and McCann, 1976; Rivier and Vale, 1978). However, there is no direct evidence of the cellular localization of histamine in the pituitary gland.

Peripheral histamine is not located exclusively in classic mast cells (Code, 1977). The present report supports the concept that histamine is located in peripheral neurons and endocrine cells in addition to mast cells.

Material and Methods

Antisera against histamine coupled to succinylated hemocyanin were produced in rabbits and characterized by standard radioimmunooassay procedure, solid phase immunoadsorption, and immunohistochemical blocking controls (Panula et al., 1984 a,b).

Normal adult male Sprague-Dawley rats (inbred strain of the Department of Anatomy, University of Helsinki), body weight 250–350 g, and male albino guinea pigs (Orion Laboratories, Tuohlampi, Finland) were used in this study. Eight rats each received a single intraperitoneal injection of colchicine (Sigma, St. Louis, USA; 10 mg/kg

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body weight) 48 hours before killing. Ten rats and five guinea pigs received a single injection of l-histidine (Sigma) intraperitoneally 1 hr before killing. All animals were perfused through the left ventricle with 0.9% saline followed by ice-cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, under sodium pentobarbital anesthesia. The tissues were dissected and immersed in the same fixative for another 2 hr and then transferred to 0.1 M sodium phosphate buffer, pH 7.4, containing 20% sucrose for at least 24 hours before sectioning with a cryostat or vibratome. In preliminary studies, 0.1–0.5% glutaraldehyde was included in the fixation solution, but this was found to inhibit the detection of tissue-bound histamine by the antisera and glutaraldehyde was thereafter omitted completely.

Cryostat sections (thickness 10-30 μm) were collected on gelatin-coated slides and air-dried for 2 hr. They were then rehydrated in phosphate-buffered saline containing 0.25% Triton X-100 (PBS-T; pH 7.4) for 10 min. Specific histamine antiserum was then applied at dilution 1:500 in PBS-T and the incubation was carried out at 4°C for 48 hours. The slides were washed for 20 min in PBS-T and incubated in fluorescein isothiocyanate-conjugated swine antirabbit immunoglobulins (DAKO, Copenhagen, Denmark) diluted 1:80 in PBS-T for 1 hr at room temperature. The sections were then washed in PBS without Triton X-100 for 20 min and mounted with PBS/glycerol (1:2) for examination under a Leitz Dialux fluorescence microscope equipped with an epiluminator. Consecutive sections were incubated with histamine antiserum preabsorbed with histamine, l-histidine, telemethylhistamine, β-allyl-L-histidine, L-histidyl-L-leucine, thyrotropin-releasing hormone (pGlu-His-Pro-NH₂), serotonin, norepinephrine, or epinephrine (all from Sigma), at 5, 50, or 500 μM. Histamine (5–50 μM) abolished the immunoreaction obtained with all histamine antisera from mast cells, nerves, and endocrine cells, whereas the other substances except for telemethylhistamine were ineffective even at the highest concentration. Telemethylhistamine (500 μM) diminished the intensity of the immunoreaction.

Results

Stomach

Brightly fluorescent histamine-containing cells were seen in the basal parts of the gastric glands in the guinea pig stomach (Figure 1A). Mast cells were also observed in different layers of the guinea pig stomach (Figure 1B). In addition, varicose nerve fibers in the guinea pig stomach were found in the external muscle layer (Figure 1C–D).

A large number of enterochromaffin-like cells in the stomach of the rat exhibited histamine immunofluorescence (Figure 2A). The immunofluorescence was completely abolished when the antiserum was preabsorbed with histamine. The cells were located in the lower parts of the gastric glands. Those parts of the glands that were close to the luminal surface were devoid of histamine-immunoreactive cells. In the superficial parts of the gastric villi, few mast cells were detected in the lamina propria. Nonspecifically fluorescent cells, probably plasma cells, were frequently found in the lamina propria. They were easily identified because their fluorescence was not abolished by preabsorption by histamine.

Adrenal Gland

Numerous histamine-containing mast cells were found outside the capsule of the adrenal gland of the rat and guinea pig (Figure 2B). In the guinea pig, but not in the rat, numerous small process-bearing cells in the adrenal medulla exhibited bright granular histamine immunofluorescence (Figure 2C). Some of these cells may correspond to the small granule-containing cells or neurons of the adrenal medulla, because they were seen to send out long processes. Most cells, however, had the morphology of mast cells, with granular fluorescence and irregular shape. Preabsorption with histamine completely abolished the reaction (Figure 2D). Preliminary electron microscopic studies have confirmed the intracellular cytoplasmic location of the immunoreactivity.

Intestine

A sparse network of immunoreactive nerve fibers was detected in the mucosa of the ileum with all antisera (Figure 3A and C). Preabsorption of the antiserum with histamine abolished all immunofluorescence (Figure 3B). The fibers were most numerous immediately under the epithelium (Figure 3C). Considerably fewer fibers were found in the jejunum. Loading of the rats l-histidine increased the intensity of the fluorescence and the number of immunoreactive fibers. A relatively dense network of immunoreactive fibers was found in the submucosal plexus of the ileum, whereas few fibers were seen in the myenteric plexus. The nerve cells in the ganglia of the myenteric plexus did not contain histamine even after loading of the animals with l-histidine or intraperitoneal injection of colchicine, although a punctate pattern of immunofluorescent terminals was detected around these cells (Figure 3D). In contrast, immunofluorescent nerve cells were seen frequently in the submucosal ganglion cell layer after intraperitoneal colchicine treatment. In order to find the origin of the histamine-immunoreactive fibers in the ileum, several normal and l-histidine-loaded animals were analyzed for possible histamine-immunoreactive sensory neurons in the spinal ganglia. Only immunoreactive mast cells were seen in these ganglia (data not shown).

Histamine-immunoreactive endocrine cells were seen in the glands of the ileum and jejunum (Fig. 3A and E) of the rat. Loading of the animals with l-histidine increased the intensity of the fluorescence in these cells but did not affect the number of fluorescent cells. These cells were always located in the glands and exhibited bright granular fluorescence which was most intense in the basal parts of the cells.

Histamine-immunoreactive mast cells were commonly detected in the lamina propria of the rat ileum (Figure 3F).

Pituitary Gland

No histamine-immunoreactive nerve fibers were observed in normal or histidine-loaded male rat or guinea pig pituitary
Figure 1
HISTAMINE IN PERIPHERAL NERVES AND ENDOCRINE CELLS

Figure 2. (A) Histamine-immunoreactive enterochromaffin-like cells in the oxyntic gland area of the rat stomach. The cells are located in the basal parts of the glands. cm = Circular muscle layer, sm = submucosal layer. Original magnification × 100. Bar = 100 μm. (B) Brightly histamine-immunofluorescent mast cells outside the cortex (c) of the guinea pig adrenal gland. zg = Zona glomerulosa. Original magnification × 250. Bar = 100 μm. (C) Numerous irregular cells with processes in the guinea pig adrenal medulla exhibit histamine immunofluorescence between groups of nonreactive chromaffin cells. Original magnification × 400. Bar = 50 μm. (D) When the histamine antiserum was preabsorbed with 50 μM histamine no fluorescence was seen in the guinea pig adrenal medulla. Original magnification × 400. Bar = 50 μm.

Gland. Histamine-containing granular mast cells were seen regularly in the neural lobe (Figure 4).

Discussion

This study provides histochemical evidence for the presence of histamine in peripheral nervous and endocrine systems in addition to mast cells. It appears that the distribution of histamine resembles that of serotonin: Both are present in mast cells of certain species, in neurons and endocrine cells in the gastrointestinal tract, and in a small population of neurons in the brain (Panula et al., 1984a,b).

Histamine is present in neurons of the posterior hypothalamic region of the rat (Panula et al., 1984a,b), and it stimulates the secretion of some anterior pituitary hormones (Weiner and Ganong, 1978). The lack of histamine-immunoreactive neuronal fibers in the pituitary gland suggests that direct participation of histaminergic neurons in the regulation of this gland is unlikely. Moreover, this finding is in agreement with the idea that histamine participates in the physiological regulation of secretion of the pituitary hormones, for example ACTH (Dallman and Yates, 1968; Cowan, 1975; Weiner and Ganong, 1978), in an indirect way (Ganong, 1963), perhaps by releasing vasopressin, which has corticotropin releasing hormone (CRH) activity (Dogterom et al., 1976). The median eminence has the highest histamine content in the hypothalamus (Brownstein et al., 1974; Pollard et al., 1976), but the histidine decarboxylase content is low (Pollard et al., 1976), suggesting that histidine in the median eminence is located in mast cells, which are known to have a low enzymatic activity. This inference is supported by immunohistochemical demonstration of histamine-containing mast cells but few nerve fibers in the median eminence (Panula et al., 1984b), and an increase instead of a decrease in the histamine content of the posterior pituitary after chronic stalk sectioning in the rat (Verburg et al., 1983). The presence of histamine-immunoreactive enterochromaffin-like cells in the gastric mucosa is in agreement with earlier results obtained with the o-phthalaldehyde method (Juhlin and Shelley, 1966; Håkanson and Öwman, 1967). Our results are, therefore, in agreement with recent results obtained with antibodies against L-histidine decarboxylase (HDC), which have shown immunoreactivity in enterochromaffin-like cells in the basal parts of the oxyntic glands in the rat stomach (Taguchi et al., 1984; Kubota et al., 1984).

We were not able to find histamine immunoreactivity in the parietal cells, which contained HDC-like immunoreactivity in an earlier study (Tran and Snyder, 1981). Our results indicate that the immunohistochemical method is specific for histamine and does not detect other related substances except for tele-methylhistamine, which is a metabolite of histamine and therefore does not cause major problems in the interpretation of the results. Therefore, the method is suitable for studying the relations between cells containing histamine, catecholamines, and peptides in the stomach. This is important, because the exact role of histamine in the regulation of gastric secretion is not yet clear (Soll and Walsh, 1979). However, it is known that histamine stimulates gastric secretion of highly acidic fluid (Ivy and Bachrach, 1966) and acts in collaboration with cholinergic vagal efferents and gastrin (Soll and Walsh, 1979). The histamine-immunoreactive cells were located in the basal parts of the gastric glands in the oxyntic gland area of the rat stomach in agreement with earlier studies (Håkanson and Öwman, 1967; Thunberg, 1967).

Histamine was located in three different cellular compartments in the rat ileum and guinea pig stomach. Rat mast cells, which were found in the lamina propria, are known to contain histamine. There is pharmacologic evidence for histamine-containing nerves in the gut (Håkanson et al., 1983) but they have not been demonstrated histochemically before. Endocrine cells in the ileum contain a number of peptides and catecholamines, but previous studies with the o-phthalaldehyde method have not revealed these cells. It is not known at present whether histamine is synthesized in these cells or is taken up by the cells and produced elsewhere, for example by mast cells. Histamine is present in peripheral nerves (von Euler, 1956; MacDonald et al., 1981; Ryan and Brody, 1970), but the presence of mast cells in nerve trunks has made it difficult to estimate the possible role of neuronal histamine (Olsson, 1968; Torp, 1961). Our results show the presence of histamine in subepithelial nerves of the rat ileum and the origin of at least some of the histamine-containing fibres in the submucous ganglion cell layer. The histamine-containing nerves were present but sparse in the myenteric plexus, and the cells in this layer remained nonreactive even after colchicine pretreatment of the animals, which suggests that the nerves do not originate in this layer. The general effect of histamine on the ileum is contraction (Douglas, 1980). The appearance of immunoreactive histamine in the ganglion cells of the submucous ganglion cell layer after colchicine pretreatment suggests but does not prove that histamine is synthesized in these cells, and not only taken up by the nerves from the extracellular space. The intrinsic gut neurons that originate in the submucous plexus contain substance P, vasoactive intestinal polypeptide, somatostatin, and cholecystokinin (Schultzberg, 1983). Of these peptides, substance P causes atropine-resistant contractions of the gut (von Euler and Gaddum, 1931; Pernow, 1953; Yau, 1978). It remains to be studied whether histamine is located in the same cells as these peptides or is released by them. Studies on isolated strips of stomach wall and taenia coli have shown that histamine can be released by gastrin and cholecystokinin (Håkanson et al., 1983). In our studies the
Figure 3. (A) A cross section of the glands of the rat ileum shows two endocrine cells (arrowheads) and a nerve fiber (arrows) that show histamine immunofluorescence. Original magnification ×250. (B) When the histamine antiserum was absorbed with 50 μM histamine, no immunoreactive cells or nerves were detected in the rat ileum. Original magnification ×250. (C) A longitudinal section of the villi in the rat ileum shows subepithelial nerves that contain histamine. Original magnification ×400. (D) Fine varicose fibers and terminal-like structures exhibit histamine immunofluorescence in the myenteric plexus between the longitudinal (m) and circular (cm) muscle layers and in the submucous ganglion cell layer (arrows) of the rat ileum. Original magnification ×400. (E) A single endocrine cell in the rat ileum shows granular histamine immunofluorescence. Original magnification ×400. (F) Mast cells in the lamina propria of the rat ileum exhibit histamine immunofluorescence. Original magnification ×400. Bars = 50 μm.

Figure 4. (A) Bright histamine immunofluorescence in mast cells of the rat posterior pituitary. No immunofluorescent nerve fibers are seen. Original magnification ×250. Bar = 100 μm. (B) Mast cells exhibiting histamine immunofluorescence in the rat posterior pituitary are irregular in shape and contain granules typical of mast cells. Original magnification ×400. Bar = 50 μm.

Spinal sensory ganglia contained only histamine-immunoreactive mast cells whereas the neurons were nonreactive (data not shown). Our studies on the sympathetic ganglia of the abdominal region of the rat and guinea pig have not revealed histamine-immunoreactive principal ganglion cells, although immunoreactive SIF (small intensely fluorescent) cells were detected (Häppölä et al., 1985).

Surprisingly, numerous brightly fluorescent, process-bearing histamine-immunoreactive cells were found in the adrenal medulla of the guinea pig but not the rat. Morphologically these cells did not correspond to the chromaffin cells, and they were also found to be different from the cells that are found immunohistochemically to contain the epinephrine-synthe-
sizing enzyme phenylethanolamine-N-methyltransferase, or by the formaldehyde-induced fluorescence method to contain norepinephrine (data not shown). The guinea pig adrenal medulla has small granule-containing cells and two types of neurons in addition to the epinephrine and norepinephrine-storing cells (Unsicker et al., 1978). The identification of these histamine-immunoreactive cells is difficult at the light microscopic level, because the mast cells in guinea pigs may also send processes.

The nature of these cells must therefore be studied by electron microscopic methods. Preliminary studies suggest that histamine is also present in adrenal chromaffin cells of certain species (unpublished observations). Histamine can depolarize the adrenal chromaffin cells (Douglas et al., 1967) and release catecholamines from these cells (Staszewska-Barczak and Vane, 1965). The effect of systemically administered histamine on the blood pressure varies in different species. In the rat, the response depends on the strain and season of the year (Fearn et al., 1966), whereas a triphasic response has been observed in the guinea pig (Levi et al., 1975). It might be of interest to study the possible correlations of the histamine-containing cells and histamine receptors in the adrenal gland and the effects of histamine on the blood pressure.

It is obvious that the histamine-containing neuronal and endocrine systems may be more widespread than revealed by this study. Further studies will show whether the differences observed between the distribution of histamine-containing cells in the rat and guinea pig demonstrated in this study are significant. It is possible that the detection limit of the immunohistochemical method does not allow visualization of all histamine-containing cells. Furthermore, these studies must be accompanied by parallel experiments with the antibodies against histidine decarboxylase, the histamine-synthesizing enzyme.

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