REVIEW

Histamine, mast cells and ovarian function

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RECEIVED 29 June 1988

ABSTRACT

Mast cells, endothelial cells, basophils and platelets are potential sources of histamine in the ovary. Little is known about the role of the latter three cell types in ovarian function. Several studies have revealed changes in the number and degranulation (release of histamine) of mast cells in the ovary during the cycle. Mast cells degranulate on pro-oestrus in the rodent ovary, and mast cells numbers increase in the theca externa of the dominant follicle in the bovine ovary. In rodents, mast cells are limited to the ovarian hilum and are not observed in follicles, corpora lutea and interstitium; this contrasts with larger species such as man, cows and monkeys where mast cells are observed throughout the ovary. Evidence is accumulating that mast cell degranulation in the ovary may be regulated by neuronal input. Neurones have been shown to have close morphological relationships with mast cells in the ovary. Histamine participates in regulating capillary permeability and blood flow in the ovary. These actions are induced by injections of LH, yet the mechanism by which LH induces mast cell degranulation is unknown. Histamine stimulates ovarian contractility, ovulation and follicular progesterone secretion in vitro. Whether these actions of histamine occur in vivo are currently unknown. This review gives a chronological description of the discoveries of the effects of histamine on ovarian function and makes suggestions for future research in this area.

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Cellular sources of histamine in the ovary

There are four well-known cellular sources of histamine: mast cells, basophils, platelets and endothelial cells (Schayer, 1962; Beaven, 1978), and they may be potential sources of histamine in the ovary. Mast cells and endothelial cells are present within ovarian tissues, are relatively stationary and thus do not normally circulate as do basophils and platelets. In mammals, histamine may exist bound to heparin or heparin-like molecules in granules of the mast cells, basophils and platelets (Beaven, 1978), whereas endothelial cells within blood vessels of all sizes contain histamine but apparently not in the granular form. Capillary endothelial cells during angiogenesis migrate into tissues (Meyer & Bruce, 1980) and thus carry within the migrating cells histidine decarboxylase, the enzyme that converts histidine to histamine (Schayer, 1962). The changes in the cellular sources of histamine within the ovary are not well understood; however, studies from our laboratories and others have revealed ovarian alterations in histamine, and these studies serve as a basis for this review. The lack of information on ovarian endothelial cells, platelets and basophils precludes any description of their possible roles.

Mast cells in the rat ovary

Numerous mast cells have been described in the rat ovarian medulla and the connective tissues of the hilum region (Jones, Duvall & Guillette, 1980; Schmidt, Owman & Sjoberg, 1988). Mast cells were observed neither in thecal tissue of the follicles nor in corpora lutea (Jones et al. 1980). The number of mast cells in the medullary region varied with the day of the oestrous cycle, the number being highest at oestrus, intermediary at dioestrus and metoestrus and lowest at pro-oestrus. The ovarian medulla contained two to four times fewer mast cells (per unit area) than the hilum and the number of mast cells in the hilum did not change significantly throughout the cycle.

In the ovarian medulla of the rat, mast cells have been located in the connective tissues of the stroma, near venules, arterioles, lymphatics and interstitial tissues. The mast cells in the medulla (not the hilum) degranulated on pro-oestrus indicating that mast cell histamine may be involved in the ovarian hyperaemia observed at the time of the luteinizing hormone (LH) surge.

**Mast cells in the hamster ovary**

Mast cells have been observed in the hilum, in the vicinity of blood vessels that enter and leave the ovary, and in the ovarian bursal region (Krishna & Terranova, 1985). A significant increase in the percentage of extensively degranulating mast cells (>15 granules released from the cell body) was observed in the ovarian complex coincident with the LH surge on pro-oestrus. In addition, the highest concentration of histamine in the ovary on pro-oestrus coincided with the greatest percentage degranulation of mast cell. These results indicated that mast cells may be a source of the ovarian histamine observed on pro-oestrus. Most recently, mast cells have been described in the mesothelial lining of the ovarian bursa (Shinohara, Nakatani, Morisawa et al. 1987). The number of mast cells in the connective tissue area within 250 µm of the bursal mesothelium decreased on pro-oestrus when compared with the numbers on days 1–3 of the cycle. However, the numbers of mast cells in the connective tissue within 50 µm of the bursal mesothelium decreased from days 1 to 2 but were not altered on days 2, 3 and 4 of the cycle. Mast cells within 50 µm of the bursal mesothelium accounted for 40–50% of the mast cells within 250 µm of the mesothelium. Mast cell granules were occasionally observed in the bursal cavity indicating that bursal mast cells may release their products directly into this cavity and thus the secretory products of mast cells may affect ovarian function. We have also observed mast cell granules in the bursal cavity (A. Krishna & P. F. Terranova, unpublished observation).

We have ascertained the number, distribution and degranulation of mast cells in the hamster ovarian complex (ovary, ovarian blood vessels entering/leaving the ovary, fat pad, bursal region and hilum) (A. Krishna & P. F. Terranova, unpublished observations). The distribution of mast cells was not uniform. Most were located in the vicinity of blood vessels that enter/leave the ovary (42%) and in the fat pad surrounding the bursal cavity (37%). Approximately 20% of the mast cells were located near the bursal mesothelium. A very small percentage of mast cells (<1%) was observed in the ovarian hilum; mast cells were not observed in follicles and corpora lutea. The number of mast cells did not change significantly at 09.00 h on days 1 (oestrus) to 4 (pro-oestrus). At 15.00 and 21.00 h on day 4 (during and after the LH surge), the number of mast cells was lower than at 09.00 h on day 4. In regard to degranulation, the percentage of mast cells in all compartments releasing more than five granules/cell was higher at 15.00 h on day 4 than at any other time as previously reported (Krishna & Terranova, 1985). Most interestingly, there was a differential pattern of degranulation by compartment. For example, extensive degranulation (>15 granules released per cell) increased significantly from days 1 to 2 in the hilar and bursal regions but not in the blood vessel and fat pad regions. On day 4 at 09.00 h (before the LH surge), an increase in degranulation (more than five granules/cell) was observed in each compartment compared with day 3 at 09.00 h. These unpublished studies reveal that on pro-oestrus ovarian mast cells begin a synchronized degranulation before the LH surge, and that events on pro-oestrus associated with the peak of the LH/follicle-stimulating hormone (FSH) surges further increase degranulation. In addition, the results indicate that increases in degranulation on days 1–3 of the cycle can occur asynchronously and independently within each ovarian compartment.

**Mast cells in the bovine ovary**

Mast cells have also been located in the bovine ovary (Cupp, Laben & Meed, 1959; Nakamura, Smith, Krishna & Terranova, 1987). In contrast to the situation in the rat and hamster, mast cells were observed in the theca externa of follicles, the external capsule of the corpus luteum and the ovarian stroma and hilum. The largest number of mast cells was observed in the ovarian stroma and hilum. However, on day 19 of the cycle (during the late follicular phase), a significant increase in the number of mast cells was observed in the theca externa of the dominant follicle compared with smaller healthy follicles in the same ovary. A very early study has also reported that mast cells infiltrate the ovary during the latter portion of the follicular phase (Cupp et al. 1959); apparently, these migrating mast cells were originally located in the ovarian stroma. Rupture of the dominant follicle and formation of a corpus luteum was associated with a significant decrease in the number of mast cells in corpora lutea on day 4 of the cycle (early luteal phase) (Nakamura et al. 1987). This is not unlike the situation in the rat and hamster, where pro-oestrous LH and FSH surges are associated with an increase in mast cell degranulation and subsequent reduction in the number of ovarian mast cells (Jones et al. 1980; Krishna & Terranova, 1985).

**Ovarian mast cells in other species**

We have also observed mast cells in the ovary of man, rhesus monkey, pig and mouse using the quick
Toluidine blue method (Humason, 1962). Mast cells were not observed within the ovary of the sheep, guinea-pig and rabbit using the same method. In contrast, Stefenson, Owman, Sjoberg et al. (1981) using fluorescence have observed a granular type of fluorescent cell (mast cell) in the ovary of the sheep and guinea-pig. The reason for these differences is unknown. In man (Jaiswal, Krishna & Pandey, 1987), rhesus monkey and pig, mast cells were observed in the stromal, hilar, luteal and external thecal regions, similar to the cow (Nakamura et al. 1987). In the mouse, mast cells were limited to the bursal, hilar and mediullary regions of the ovary, similar to the rat and hamster (Jones et al. 1980; Krishna & Terranova, 1985); mast cells were not observed within follicles and corpora lutea. The reasons for the differences in location of the mast cells in the larger species (cow, man, pig and monkey) compared with the smaller species (hamster, rat and mouse), and the reason for the absence of mast cells in the ovary of the guinea-pig and rabbit are unknown.

A recent study has shown that the human dermal mast cell population exhibits heterogeneity with respect to fixation with 10% neutral buffered formalin, or with Carnoy's fluid, and subsequent staining with Alcian blue/safranin and Toluidine blue (Marshall, Ford & Bell, 1987). Significantly more mast cells were observed with Alcian blue/safranin than with Toluidine blue and, using both stains, only 50% of the mast cells observed in specimens fixed in Carnoy's fluid were observed in tissue fixed in formalin. Thus, in the human dermis, some mast cells do not stain with Toluidine blue after being fixed in formalin. We have fixed hamster ovaries in Carnoy's fluid and subsequently stained the paraffin-embedded cut sections with Toluidine blue. The distribution of mast cells remained the same as in formalin-fixed ovaries. Neither strict quantitative measurements nor staining with Alcian blue/safranin of the hamster ovary has been performed. A characteristic of rat intestinal mast cells (mucosal mast cells) is that after fixation in formalin their capacity to stain with Toluidine blue is lost or greatly reduced, whereas mast cells from the peritoneum stain well under identical conditions of fixation. We have observed very 'faint' staining populations of mast cells among darker staining mast cells in the ovaries of the cow, pig and man, but not in rodents. Thus, the possibility exists that ovaries from the large species contain heterogeneous populations of mast cells that lose their ability to stain during formalin fixation. It is also likely that the length of formalin fixation may be a factor in staining with Toluidine blue (Marshall et al. 1987).

Neuronal-mast cell interactions in the ovary
A study by Walles, Edvinsson, Falck et al. (1975) revealed mast cells in the theca externa of the bovine ovary; these cells exhibited green fluorescence, indicating the presence of dopamine, and were intermingled amongst the adrenergic nerves. A later study by Stefenson et al. (1981) also reported fluorescent ovarian mast cells in the cow, sheep, rat, guinea-pig and opossum intermingled amongst a neuronal network. In the cow, sheep and opossum, mast cells were observed within theca externa in association with adrenergic neurones and these mast cells emitted a green fluorescence indicative of dopamine. In guinea-pig and rat ovaries, a yellow fluorescent-type of stromal mast cell was observed indicating the presence of serotonin. Whether these neurones interact with mast cells in the regulation of ovarian function is unknown, but it has been suggested (Schmidt, Owman & Sjoberg, 1986). Electron micrographs have also revealed a close morphological relationship between non-myelinated nerves and mast cells in subungual tumour tissue (Wiesner-Menzel, Schulz, Vakilzadeh & Czarnetzki, 1981). Mast cells containing immature granules with numerous lamellopodia were in direct contact with nerves, whereas those with mature granules with few lamellopodia were within a distance of 20–2000 nm of the nerve. Using immunocytochemistry, peptidergic nerves containing substance P and vasoactive intestinal peptide (VIP) have been observed in close proximity to the ovarian hilar mast cells of the hamster; in addition, substance P (Nakamura & Terranova, 1987) and VIP induce mast cell degranulation in vitro (P. F. Terranova, Y. Nakamura & B. K. Gangrade, unpublished observations). The mechanism by which neurones are activated possibly to interact with mast cells in regulating ovarian function is unknown.

Relationship between histamine and ovarian function
Effects of histamine on blood flow and follicular development
Ovarian hyperaemia has been used as an indicator of the action of pituitary LH and chorionic gonadotrophin for many years (Eberon & Silverberg, 1931). In fact, Ellis (1961) established a bioassay of LH using an increase in content of radioiodinated serum albumin as an index of ovarian hyperaemia. Schayer (1962) recognized that free histamine may regulate ovarian hyperaemia induced by LH. It then became well established that ovarian oedema and hyperaemia occur within minutes after i.v. injection of LH (or human chorionic gonadotrophin (hCG) and that this was accompanied by a depletion of ovarian histamine (Szego & Gitin, 1964). An increase in ovarian water occurred by 1 h (earliest time measured) and a peak in 125I-labelled albumin uptake occurred in the ovary between 2 and 2.5 h after injection of LH indicating increased permeability (Szego & Gitin, 1964; for
review see Szego, 1965). Wurtman (1964) reported that LH induced a rapid increase in the proportion of cardiac output delivered to the ovaries as measured by the ovarian uptake of $^{42}$K. Ovarian blood flow increased by 75% 20 min after injection of LH and this effect was mimicked by histamine, but not by prolactin, serotonin, epinephrine or norepinephrine. The latter two studies represented the first of several studies implicating histamine as a mediator of the action of LH on ovarian function.

Varga, Acs, Papp & Stark (1967) investigated the effects of histamine on ovarian blood flow in the dog. Ovarian blood was measured in chloralose-anaesthetized dogs using a flexible thermocouple which measured changes in heat conduction associated with changes in blood flow. Histamine (25 μg/kg per min) given i.v. increased ovarian blood flow to twice that of initial values within 5 min of infusion, whereas 50 μg/kg caused a significant decline in ovarian blood flow. The results, although somewhat ambiguous, indicated that histamine influences ovarian blood flow and that biphasic effects of this biogenic amine may occur. The reproductive status of the dogs was not given.

Hunter & Leathem (1968) hypothesized that the marked accumulation of follicular fluid in cystic ovaries of hypothyroid rats may have been due to increased release of histamine. Induction of hypothyroidism, by feeding rats 0·5% thiouracil, reduced the total amount of ovarian histamine and this effect occurred more acutely in rats receiving daily injections of hCG. Thus, it was concluded that histamine may have been involved in the development of cystic follicles.

The next studies relating LH and histamine were those of Lipner (1971) and Piacsek & Huth (1971). Lipner (1971) reported that 30 IU pregnant mare serum gonadotrophin (PMSG) increased circulating levels of histamine from 0.1 μg/ml blood to 0.15 μg/ml and subsequent injection of the same dose of hCG increased blood levels of histamine to 0.225 μg/ml. Compound 48–80, which causes mast cell degranulation and depletion of the stores of histamine in mast cells, prevented the PMSG- and hCG-induced increase in blood levels of histamine, drastically reduced ovarian histamine from 7·5 ng/mg ovary to 2.0 ng/mg ovary and reduced ovarian weight from 65·6 ± 4·6 mg (PMSG-primed) to 36·2 ± 4·8 mg (Compound 48/80- and antihistamine-treated groups combined). Benadryl, an H1 receptor blocker, greatly reduced ovarian hyperaemia. These studies indicated that blockage of the action of histamine, or depletion of histamine-reduced ovarian responsiveness to PMSG and hCG, prevented increased ovarian hyperaemia and reduced ovarian weight. A reduction in the number of ova shed was also observed in the antihistamine-treated animals and could have been due to altered follicular development and/or prevention of the rupture of preovulatory follicles. Corroborative data from our laboratory (Krishna, Terranova, Matteri & Papkoff, 1986) supports and extends the findings of Lipner (1971). Constant infusion of LH throughout the oestrous cycle of the hamster beginning on day 1 (oestrus) revealed an increase in ovarian blood flow by day 3, reduced ovarian histamine concentrations and stimulated a superovulation of 30 ova at the next expected oestrus. Treatment with α-fluoromethylhistidine, an irreversible inhibitor of histidine decarboxylase (converts histidine to histamine), or cimetidine (an H2 blocker) reduced the number of ova shed in the LH-treated hamsters. The reduction in the number of ova shed was probably due to a reduction by the antihistamine in the number of follicles recruited into the developing pool, and not due to prevention of follicular rupture since no unruptured Graafian follicles were observed on oestrus in the groups treated with LH plus antihistamine. Thus, it is highly likely that the increase in ovarian blood flow induced by continuous LH treatment was prevented by the antihistamine; this would reduce the amount of LH and FSH reaching the ovary and prevent the ‘normal’ superovulatory response to the exogenous LH.

A study by Piacsek & Huth (1971) revealed that the H1 receptor blocker, promethazine hydrochloride, prevented an LH-induced increase in ovarian venous blood flow in the rat. Anaesthetized (sodium pentobarbital) cyclic rats in dioestrus were given an i.v. injection of ovine LH and/or the antihistamine (50 μg/kg body wt) 20 min before cannulation of the ovarian vein. In controls, ovarian venous blood flow decreased to 38% of initial values by 19.5 min. Administration of LH promptly increased the blood flow rate to 123% of the initial values by 4·5 min and at 10·5 min flow was still at 100%. At 19·5 min, blood flow declined to 63% of initial values, unlike control values at 38%. Promethazine hydrochloride prevented the LH-induced increase in ovarian venous blood flow; in fact, the values in antihistamine-treated animals were not different from controls. Thus, the H1 blocker prevented an LH-induced increase in ovarian blood flow.

Effects of histamine on follicular rupture
Knox & Beck (1976) and Knox, Lowry & Beck (1979) examined the possibility that the H1 blocker, chlortri-meton (10 mg/kg given i.m.), could inhibit ovulation. Female rabbits were injected every 4 h with the antihistamine for 4 days. After 2 days of treatment, an ovulation-inducing dose of hCG was administered. Appropriately injected controls exhibited ten corpora lutea/animal 10 days after injection of hCG, whereas

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chlortrimeton-treated rabbits exhibited only one corpus luteum/animal. Similar results were obtained using mating rather than hCG as the ovulatory stimulus. Numerous large haemorrhagic follicles containing ova were observed in the antihistamine-treated rabbits, indicating a blockade of follicular rupture. In addition, progesterone levels in sera were less in antihistamine-treated rabbits than in controls. This did not necessarily indicate disruption of luteal function since fewer corpora lutea were observed in antihistamine-treated rabbits. Similar results were obtained by Wallach, Wright & Hamada (1978) using rabbit ovaries perfused in vitro with the H₁ block, chlorpheniramine, but given an ovulation-inducing dose of hCG in vivo. Ovaries were removed 2, 4, 6 and 8 h after administration of hCG and placed in a perfusion chamber. Chlorpheniramine was added to the perfusion medium of one ovary; the remaining ovary serving as a control. Ovulation occurred in all control ovaries. When chlorpheniramine was in the perfusing medium, 2–4 h after hCG, ovulation was blocked in seven out of ten rabbits. Ovulation was not blocked when chlorpheniramine was added to the medium 6–8 h after hCG was given to the animals. Thus, as the interval between administration of hCG to the rabbit and addition of the antihistamine to the perfusate was increased, the frequency of ovulation approached control values, i.e. the antihistamine was ineffective in blocking ovulation. In other experiments, chlorpheniramine and hCG were added to perfused rabbit ovaries. In all cases, the number of ovulations was reduced. Thus, chlorpheniramine blocked or reduced ovulation in vitro whether hCG was given in vivo or in vitro. Most interestingly, prostaglandin F₂α given with the chlorpheniramine in the perfusate overcame the inhibitory effect of the antihistamine in rabbits given hCG in vivo. Although the mechanism by which the antihistamine blocks ovulation is unknown, it was noted by Wallach et al. (1978) that ovarian contractions were greatly reduced by treatment with antihistamine. Thus, the H₁ block, chlorpheniramine, may prevent ovulation by blockade of histamine-induced ovarian contractions which may be causally related to expulsion of the oocyte.

Espey, Stein & Dumitrescu (1982) reported that hCG- induced ovulation in the rabbit was not inhibited by chlorpheniramine, diphenhydramine or cimetidine. This discovery was different from the studies of Knox & Beck (1976) and Knox et al. (1979), who found that antihistamine blocked ovulation. Several notable differences existed between the two studies. First, Knox & Beck (1976) administered the antihistamines every 4 h for 4 days and an ovulating dose of hCG (100 IU/kg i.v.) was given at 2 days, which was the midpoint of the antihistamine treatment. Espey et al. (1982) administered multiple injections of antihistamine at 4-h intervals, beginning 1 h before the injection of the ovulating dose of hCG (50 IU/kg i.v.). Possibly, the earlier 2-day pretreatment with the antihistamine as given by Knox & Beck (1976) was needed to block the ovulatory action of hCG effectively. On the other hand, Espey et al. (1982) indicated that Knox failed to confirm inhibition of ovulation; they concluded from indirect evidence by counting corpora lutea and fetuses 10 days after administration of the antihistamine. Espey et al. (1982) suggested that the large doses of antihistamine given by Knox may have interfered with luteal and follicular development. In fact, a recent report by Halterman & Murdoch (1986) has shown that cimetidine injected directly into preovulatory sheep follicles did not inhibit ovulation but caused a reduced amount of luteal progesterone secretion during the subsequent early luteal phase. In addition, it was suggested by Espey et al. (1982) that the reason antihistamines are not effective anti-inflammatory agents is that they act only as competitive antagonists of histamine rather than as inhibitors of histamine formation within cells. We have found that α-fluoromethyl histidine (αFMH), a competitive inhibitor of the enzyme histidine decarboxylase (converts histidine to histamine), reduces the superovulatory response (follicular development) to LH in the hamster (Krishna et al. 1986); however, we have not attempted to block follicular rupture with this compound. If Espey et al. (1982) are correct, then αFMH may be very effective in inhibiting ovulation.

Additional experiments have been performed to elucidate further the role of histamine in follicular development and rupture, and ovum maturation (Kobayashi, Wright, Santulli et al. 1983). Perfused rabbit ovaries exposed to histamine (10 and 100 ng/ml perfusion medium) in vitro ovulated; however, the percentage of mature follicles that rupture (called ovulatory efficiency) was 40%, which was about half that of ovaries exposed in vitro to 50 or 100 IU hCG. When 1 ng histamine/ml was used, ovulatory efficiency was <20%. Cimetidine, an H₂ blocker, but not chlorpheniramine, an H₁ blocker, effectively blocked the ‘histamine-induced’ ovulation. Neither drug alone nor in combination blocked the ‘hCG- induced’ ovulations in vitro. The processes of enlargement and rupture of preovulatory follicles with histamine treatment were morphologically different from those with hCG treatment. The preovulatory follicle of histamine-treated ovaries lacked a stigma that was well-formed in hCG-treated ovaries. It was also noted that hCG-treated ovaries projected the ovum out of the centre of the follicle and away from the follicular surface; a histamine-induced ovulation was characterized by a disruption at any location on the follicular surface. Thus, rather than expelling the
ovum from the follicle as in hCG-induced ovulation, the ovum 'floated' away from the surface of the ruptured follicle. Since antihistamines were unable to block hCG-induced ovulation in vitro, it appears that histamine is not necessary for in-vitro ovulation; although its importance as a mediator in the ovulatory process cannot be overlooked, especially since significant alterations in ovarian histamine concentrations occur during the periovulatory period.

In the rat, the effects of LH, histamine and antihistamines on in-vitro ovulation have been investigated using perfused ovaries (Schmidt et al. 1986, 1988). In vitro, LH and histamine, each alone, caused ovarian oedema and ovulation. LH induced seven ovulations per ovary, whereas histamine induced 2-3 ovulations per ovary; all of the LH- or histamine-treated ovaries ovulated, thus the percentage of ovaries ovulating was the same (100%) in both groups. Pyrilamine (0-1 mmol/l) or cimetidine (0-5 mmol/l), H₁ and H₂ receptor blockers respectively, completely inhibited the histamine- induced ovulation, but at 10 μmol/l these treatments were ineffective. In the presence of LH, pyrilamine at the high dose (0-1 mmol/l), but not cimetidine (0-5 mmol/l), tended to reduce the number of ovaries ovulating compared with ovaries treated with LH alone. Both drugs alone tended to reduce the number of ova shed compared with those receiving only LH, and in combination they significantly reduced the number of ovulations/ovary (2-4 ova) compared with LH (7-0 ova). Indomethacin did not significantly reduce the number of ovulating ovaries, although it tended to; only one dose of indomethacin (10 μg/ml suspension) being tested. Whether higher doses of indomethacin would be effective in blocking histamine-induced ovulation in vitro is not known.

Effects of histamine on follicular contraction
Several studies have shown histamine as a mediator of ovarian and follicular contractility in rabbits, man and cows. In the rabbit, ovarian contractions were monitored during perfusion (Wallach et al. 1978). A regular contractile pattern of one contraction/10 s was observed in ovaries at 6 h after hCG administration in vivo. Contractile activity greater than 500 mg was completely diminished to less than 100 mg at 6 h after hCG administration when chlorpheniramine (an H₁ blocker) was placed in the perfusion medium; in fact, chlorpheniramine prevented contractions immediately upon addition to the perfusate. It is well established that smooth muscle contraction is a calcium-dependent process and that smooth muscle cells are present throughout the ovarian stroma and in the preovulatory ovarian follicular wall (theca). EDTA, a calcium chelator, added to the perfusate reduced the availability of calcium for contractile activity and inhibited ovulation. Thus, chlorpheniramine may be involved in inhibition of calcium movement into the smooth muscle cells activating contraction.

In women, strips of ovarian tissue were excised from the walls of mature follicles or the capsules of corpora lutea and contractility in vitro was measured using a pressure transducer (Morikawa, Okamura, Tanenaka et al. 1981). Ovarian tissues were classified as early and late follicular, ovulatory, early and late menstrual and premenstrual. Histamine-induced contractions in vitro were higher at the late follicular and ovulatory phases than at any other phase of the cycle. This coincided with increased histamine-induced concentrations in the ovary during the late follicular and ovulatory phases. Acetylcholine induced contractions similar to histamine. It appeared that increased histamine concentrations in the ovary at the time of ovulation may be causal in inducing ovarian/follicular contractions; whether the sensitivity of the ovary to histamine increases during the late follicular and ovulatory phase is, however, unknown.

In the cow, strips of follicular wall from Graafian follicles in Krebs–Ringer buffer were connected to a force-displacement transducer in order to record isometric-displacement contractions (Schmidt, Kannisto, Owman & Walles, 1987b). With the follicle strip at its resting tension (relaxed) in Krebs–Ringer (with 4-7 mmol KCl/l), histamine-induced follicular contractions were inhibited by pyrilamine, an H₁ receptor antagonist. Most interestingly, the H₂-receptor antagonist, cimetidine, increased the sensitivity of the follicular strip to histamine by 80%. When the KCl concentration in Krebs buffer was raised to 122 mmol/l, the follicular strip became highly contracted. Under those conditions, histamine, in the presence of the H₁ antagonist, pyrilamine, caused significant relaxation of the follicle strip, indicating that H₂ receptor activation by histamine was causal in mediating the relaxation.

Ovarian histamine concentrations
Rat.
Ovarian histamine concentrations were initially measured in saline- and LH-treated immature rats by Szego & Gitin (1964). Concentrations ranged from 0-7 to 1.29 μg histamine/g fresh weight (wet weight) as determined by a modification of the method of Code & McIntire (1956) which used histamine-induced changes in contraction of the guinea-pig ileum as a bioassay. A later study by Hunter & Leatham (1968) reported ovarian histamine concentrations of 0.64 μg/g in 60-day-old rats also using the method of Code & McIntire (1956). Most recently, a study by Schmidt et al. (1988) reported ovarian histamine concentrations in the 2–5 μg/g range using
fluorometry. These levels confirmed the report of Szego & Gitin (1964) and indicated that the concentration of histamine in the ovary is similar in immature and adult rats.

Hamster.
We have also reported levels of histamine in the range of 1–5 μg/g ovary (Krishna & Terranova, 1983; Krishna et al. 1986) using a radioenzymatic assay which converts histamine to 14C-methylhistamine (Taylor & Snyder, 1972; Taylor, Krilis & Baldo, 1980). The enzyme n-methyl transferase, isolated from male mouse brain, in the presence of 14C-S-adenosyl methionine, converts histamine to 14C-methylhistamine. Significantly lower levels of ovarian histamine (17–38 ng/g) were reported by Hine, Orsini & Hegstrand (1985) using a similar radioenzymatic assay. The reasons for these differences in levels between the two laboratories are unknown at present but are being investigated in our laboratory.

Rabbit.
In the initial study by Szego & Gitin (1964), who measured ovarian histamine in immature rats using the guinea-pig ileum bioassay, it was mentioned that histamine measurements were attempted originally in rabbits. Because ovarian histamine levels were very low (<0.2–0.5 μg/g) in the rabbit ovary, rats were used. In another study, Morikawa, Okamura, Okazaki & Nichimura (1976) measured ovarian histamine in the range of 3–6 μg/g ovary in the rabbit using a fluorometric method described by Shore, Burkhalter & Cohn (1959). Thus it appears that histamine levels in the rabbit can range from <1 to 6 μg/g ovary.

Man.
Only one study has measured histamine in the human ovary (Morikawa et al. 1981) and levels of 3–10 μg/g were found.

Effect of histamine on ovarian steroidogenesis
Recent studies have shown that in-vitro perfusion of preovulatory follicles with histamine (3 mmol/l) stimulates progesterone secretion although to only a quarter of that induced by LH (Schmidt, Ahren, Brannstrom et al. 1987a). Cimetidine, an H2 antagonist, completely blocked the effect of histamine on follicular progesterone secretion. On the other hand, pyrilamine, an H1 antagonist, inhibited histamine-stimulated progesterone secretion to basal values after a transient rise for 3–4 h. The H2 agonist, 4-methylhistamine, increased follicular progesterone secretion to an extent similar to that of histamine, whereas the H1 agonist, 2-methylhistamine did not stimulate follicular progesterone secretion.

Dissociated luteal cells of the cow did not produce progesterone in response to histamine in vitro in the presence and absence of LH (Battista & Condon, 1986). Isolated hamster corpora lutea produce progesterone in response to histamine in vitro and this can be blocked by H1 and/or H2 blockers (P. F. Terranova & B. K. Gangrade, unpublished observations). The mechanism by which histamine is released to stimulate ovarian steroidogenesis may involve, in part, the mast cell degranulation induced by LH on pro-oestrus (Krishna & Terranova, 1985). Thus, LH may stimulate histamine release which synergizes with LH in promoting steroidogenesis.

Suggestions for further research
Role of peptidergic nerves in ovarian function
It has been shown that nerves containing VIP and substance P innervate the ovary (Dees, Kozlowski, Dey & Ojeda, 1985; Ahmed, Dees & Ojeda, 1986). The nerves containing VIP apparently originate from the abdominal vagus, and nerves containing substance P are from the ovarian plexus originating in the spinal cord (Ahmed et al. 1986). It is clear that the VIP stimulates ovarian steroidogenesis (Davoren & Hsueh, 1985), increases plasminogen activator production by granulosa cells (Liu, Kasson, Dahl & Hsueh, 1987) and induces mast cell degranulation and release of histamine (Shanaban, Denburg, Fox et al. 1985). On the other hand, the role of substance P is less clear; it is unable to stimulate ovarian steroidogenesis but is quite potent in stimulating mast cell degranulation, thus a role in ovarian permeability and blood flow is likely. A most intriguing area for future research is the mechanism by which the peptidergic nerves in the ovary are activated. Do gonadotrophins, steroids and prostaglandins alter the activity of these nerves? If so, what are the temporal changes in gonadotrophins and release of substance P and VIP? As preovulatory follicles enlarge, do the nerve endings sense follicular growth by compression of the thecal wall? The effects of abdominal vagotomy and/or ovarian plexus neurectomy on ovarian mast cell degranulation during pro-oestrus is of special interest. This raises the question as to whether the peptidergic nerves ‘innervate’ ovarian mast cells in the classical sense or whether the nerves release the peptides in the vicinity of the mast cells (asynaptic pathway).

Histamine and serotonin in ovarian function
Although histamine and serotonin have specific effects on ovarian permeability, contractility and steroidogenesis, the specific cell types which bind histamine and serotonin remain largely unknown. This also raises the question as to which receptor types (H1, H2 or 5-hydroxytryptamine1, 5-hydroxytryptamine2) mediate each event. Specific questions are which receptor types, cells and second messengers are
involved in stimulation of ovarian progesterone production by histamine.

Also, little information exists on the changes in ovarian concentrations of histamine and serotonin in response to gonadotrophins, during the cycle and pregnancy as well as their concentrations in specific ovarian compartments, i.e. corpora lutea, folicles, interstitial tissues, ovarian fat pad and blood vessels.

ACKNOWLEDGEMENTS

The authors thank J. Judd for her secretarial assistance and patience in typing the manuscript. This work was supported by grants from NICHD (HD20389, KO0489, HD02528) and The Council of International Exchange of Scientists.

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