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Compound 48/80-induced Degranulation of GnRH-like Immunoreactive Mast Cells in the Brain and Mesentery of the Gerbil

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Mei-Fang Yang, Chung-Liang Chien and Kuo-Shan Lu (2002) Compound 48/80-induced degranulation of GnRH-like immunoreactive mast cells in the brain and mesentery of the gerbil. *Zoological Studies* **41**(1): 99-110. Mast cells affect the extracellular milieu through the process of degranulation. Recently, mast cells in the brain of the dove and certain mammals were found to express gonadotropin-releasing hormone (GnRH)-like immunoreactivity (IR). In the present study, we examine the expression of GnRH-like IR in brain and mesenteric mast cells, and the degranulation of mast cells at these 2 sites after administration of compound 48/80 (C48/80). The gerbil, in which numerous mast cells are found in the brain and mesentery, was used as the experimental animal model. Both brain and mesenteric mast cells in control and C48/80-treated gerbils express GnRH-like IR. Mast cell degranulation was dramatic in the mesentery after C48/80 was injected intraperitoneally. Because newly extruded GnRH-like immunoreactive granules in the brain and mesentery are compact, GnRH release can be classed as a rapid-release reaction. The degranulated mast cells were restored to their normal configuration in the brain and mesentery 12 h after C48/80 treatment. We conclude that: (1) brain and mesenteric mast cells express GnRH-like IR, and (2) GnRH is released by a rapid-release reaction after C48/80 administration. http://www.sinica.edu.tw/zool/zoolstud/41.1/99.pdf

Key words: Degranulation, Mast Cell, GnRH, Brain, Mesentery.

Three types of mature mast cells are identified as serosal, mucosal, and brain with their own distinct biochemical, morphological, and functional characteristics (Theoharides 1990). Recently, gonadotropin-releasing hormone (GnRH), a neuropeptide expressed in certain neurons and nerve fibers in the rodent central nervous system (Silverman and Krey 1978, Jennes and Stumpf 1980, Witkin et al. 1982), was found to also be expressed in brain mast cells of the dove (Silver et al. 1992, Zhuang et al. 1993, Silverman et al. 1994, Silver et al. 1996), musk shrew (Gill and Rissman 1998), and mouse (Yang et al. 1999). Brain mast cells are able to secrete an array of potent mediators which may orchestrate neuroinflammation and affect the integrity of the blood-brain barrier (Purcell and Atterwill 1995). The 'cross-talk' among mast cells, lymphocytes, neurons, and glia constitutes a neuroimmune axis which is implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component, such as multiple sclerosis and Alzheimer's disease (Purcell and Atterwill 1995). The number of GnRH-immunoreactive mast cells increasing in the brain of courted male doves (Zhuang et al. 1993) and mated male mice (Yang et al. 1999) strongly suggests that brain mast cells are related to the nervous, endocrine, and immune systems (Zhuang et al. 1993, Yang et al. 1999).

Mast cells can be induced to release their secretory granules by exocytosis after various stimuli, such as certain chemical compounds (Bloom and Haegermark 1965 1967, Bloom et al. 1967, Rohlich et al. 1971), hypotonic solutions (Bloom and Haegermark 1967), and incubation with anti-IgE antibody (Chen and Enerback 1995).

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Compound 48/80 (C48/80), a basic polyamine, can induce mast cell secretion by binding to a site on the cell membrane, an event which seems to be associated with an influx of Ca^{++} into the cell (Norrby 1981). It is commonly used in in vivo experiments, and its effect on mast cells is predominant when seen at the light and electron microscopic levels. C48/80-induced mast cell degranulation can be studied either in vivo (Garden 1965, Singleton and Clark 1965, Enerback and Lundin 1974, Norrby and Enestrom 1984, Nawa et al. 1994, Zhuang et al. 1996) or in vitro (Kessler and Kuhn 1975, Kruger 1976, Nielsen et al. 1981, Takayama et al. 1994). Dvorak (1986) reported that mast cells can release granule matrix materials slowly (days) or more rapidly. The "slow-release reaction" or "piecemeal degranulation" is morphologically expressed in the progressive loss of granular materials which are transported by vesicles to the cell's surface. In contrast, stimulated mast cells can rapidly extrude granular matrix materials through multiple membrane openings to the cell's exterior, and this is regarded as a "rapid-release reaction".

It is difficult to study the function of normal mast cells in mice, because only a few mast cells exist in normal mouse brain (less than 90 mast cells/brain) (Yang et al. 1999). In our pilot studies, we found that numerous mast cells were present in the central nervous system, especially in the velum interpositum and thalamic parenchyma, and in the dorsal mesentery of Mongolian gerbils (*Meriones unguiculatus*). It has not yet been determined whether the mast cells outside the brain also contain GnRH, therefore, we used the gerbil as an animal model to examine the expression of GnRH immunoreactivity at the light and electron microscopic levels in brain and mesenteric mast cells. Moreover, in order to establish a basis for future studies on the action of mast cell granules on the extracellular milieu, we investigated the process of mast cell degranulation by treating gerbils with C48/80.

MATERIALS AND METHODS

Animals and treatments

A total of 50 adult male Mongolian gerbils (*Meriones unguiculatus*), aged from 8 to 10 wk, was used in the present study. They were housed in a temperature-controlled room (22±1 ℃) under an artificial photoperiod (L: D = 12: 12) at 55% relative humidity, with free access to food and water. Animals were injected intraperitoneally with 2.5 mg/kg body wt. of C48/80 (Sigma, St. Louis, MO, USA) in 0.85% NaCl solution. Groups of 5 injected animals were sacrificed for toluidine blue staining (2 animals) and immunoreactive labeling (3 animals), 15 or 30 min or 1, 2, 4, 6, 9, or 12 h (total n = 40) after C48/80 injection. Control animals were injected with 0.85% saline and killed 15 min (n = 5) or 2 h (n = 5) after injection.

Morphological observations

At the time of sacrifice, animals were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg body wt.), and perfused with a fixative consisting of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4.

Toluidine blue staining

The brain and mesentery of the jejunum and ileum were dissected out and fixed in the same fixative overnight. Brain coronal sections (50 μm) were cut from the anterior commissure to the rostral midbrain using a vibratome, and mounted on gelatin-coated slides. Pieces of mesentery were whole-mounted on gelatin-coated slides. All slides were air-dried, then stained with 0.125% toluidine blue, pH 2.5, at room temperature for 5 min. Mast cells were readily identifiable by their metachromatic cytoplasmic granules under a light microscope.

Immunohistochemical study

For immunohistochemical labeling, floating brain vibratome sections (50 μm) and pieces of mesentery were washed with phosphate-buffered saline (PBS), then incubated in 0.5% H_2O_2 for 30 min, followed by incubation in 0.5% bovine serum albumin and 10% normal goat serum for 1 h. Tissues were then incubated for 48 h at 4 ℃ with guinea pig anti-mammalian GnRH antibody (Protos Biotech, New York, NY, USA), or rabbit anti-LHRH antibody (DiaSorin, Stillwater, MN, USA), diluted 1: 2000 in PBS, then at room temperature for 1 h with biotinylated goat anti-guinea pig or goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA), diluted 1: 200 in PBS. They were then reacted with ABC complex (Vector Laboratories, Burlingame, CA, USA) for 1 h, washed, treated with DAB/H_2O_2 (diaminobenzidine 0.5 mg/ml, 0.02% H_2O_2 in 0.05

M Tris buffer, pH 7.6), washed, and mounted on a gelatin-coated slide with Permount (Fisher Scientific, Fair Lawn, NJ, USA). For immunofluorescent studies, specimens treated with primary antibody were incubated in a 1: 200 dilution of FITC-conjugated goat anti-guinea pig antibody (Bethyl Laboratories, Montgomery, TX, USA), washed, and mounted with crystal/mount (Biomedica, Foster City, CA, USA). After being photographed, the immunofluorescent sections were stained with toluidine blue for identification of mast cells.

In control experiments, the primary antibody was omitted or replaced with an irrelevant guinea pig antibody (guinea pig anti-GABA-B receptor antibody, Chemicon, Temecula, CA, USA). In additional specificity tests, primary antibodies were preabsorbed with 50-100 ng/ml of mammalian GnRH peptide (Peninsula Laboratories, San Carlos, CA, USA) for 24 h at 4 ℃.

Immunoelectron microscopic studies

For immunoelectron microscopic examination, DAB/H_2O_2-reacted specimens containing GnRH-like immunoreactive mast cells were post-fixed with 1% OsO_4, dehydrated, and embedded in Epon. Then ultrathin (85 nm) sections were cut, mounted on a 200-mesh grid, and examined in a JEOL 2000EX electron microscope at 100 kV.

RESULTS

Five minutes after injection of C48/80, animals showed signs of prostration and partial paraplegia. After 15 min, nasal frothing and sometimes somnolence were observed. Animals recovered the ability to move about 30 min after the injection, but movement was initially slow.

Light microscopic observations

GnRH-like immunoreactive mast cells in control animals

Brain mast cells were numerous in the velum interpositum and thalamic parenchyma. In the velum interpositum, they were present as clusters and therefore easily identifiable either by immunohistochemistry or by their metachromasia (Fig. 1A, B). The diameter of brain mast cells was 10.43±1.29 μm. In the present study, velum interpositum mast cells were used as representatives of brain mast cells.

Mast cells were evenly distributed in the mesentery. They displayed abundant granules and clear cell boundaries. The mean diameter of the mesenteric mast cells was 15.91±2.31 μm (Fig. 1C, F).

In sham-injected control animals, both brain (Fig. 1A, E) and mesenteric (Fig. 1C, F) mast cells expressed GnRH-like IR, as shown by both FITC-labeled (Fig. 1A, C) and DAB-stained (Fig. 1E, F) sections. FITC-labeled GnRH-immunoreactive cells were clearly identified as mast cells after toluidine blue staining (Fig. 1B, D).

In control sections in which the primary antibody was omitted, no immunolabeling was seen (Fig. 1G), although many mast cells could be identified by toluidine blue staining in the same section (Fig. 1H). Specificity tests involving preabsorption of the anti-GnRH antibody with mammalian GnRH showed that the staining of mast cells was markedly reduced, although not entirely eliminated. Very weak background staining was observed when an irrelevant primary antiserum (guinea pig anti-GABA-B receptor antibody) was used.

Mast cells in C48/80-treated animals

After intraperitoneal administration, brain mast cells in sham-injected control animals were shown by toluidine blue staining to be loaded with abundant purple metachromatic cytoplasmic granules and to have clear cell boundaries (Fig. 2A). In C48/80-injected animals, obvious morphological changes were seen in brain mast cells 2 h after injection, by which time brain mast cells had become degranulated with some extruded granules present in the extracellular space. Few of these extruded granules were dispersed more than about 20 μm from the cell body (Fig. 2C). The extruded granules persisted in the extracellular space for several hours, finally disappearing by about 9 h after C48/80 treatment. Degranulated mast cells were restored to their normal shape, with no extruded granules around the cell body, 12 h after C48/80 treatment (Fig. 2E).

GnRH-like IR was expressed in brain mast cells of both sham-injected control and C48/80-treated animals. In the control group, the immunoreactive products were localized in the cytoplasmic granules of mast cells (Fig. 2B). In C48/80-treated animals, 2 h after injection, GnRH-like immunoreactive granules were found both within the cell and at various distances outside the cell (Fig. 2D); while 12 h after injection, mast cells

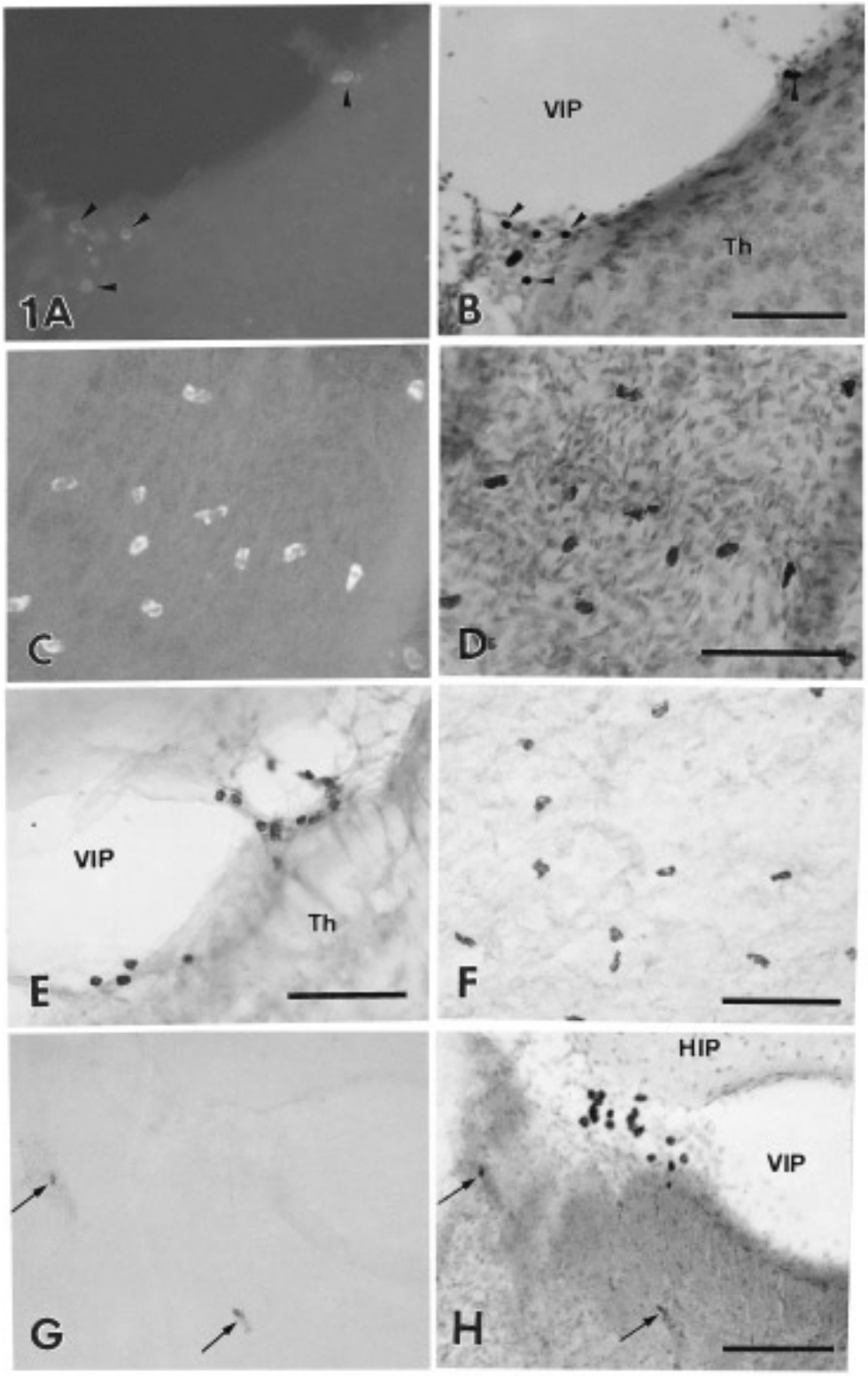

Fig. 1. GnRH-like IR in mast cells of gerbil velum interpositum (VIP) and mesentery. FITC-labeled GnRH-like IR is present in VIP mast cells (A, arrowheads) and mesenteric mast cells (C). These GnRH-like immunoreactive cells were subsequently identified as mast cells by toluidine blue staining of the same sections (B, arrowheads and D). Using the avidin-biotin method, DAB products can be seen in VIP (E) and mesenteric (F) mast cells. When the anti-GnRH antibody is omitted, no labeling is seen in the VIP area (G), although mast cell accumulation can be observed in the same section after toluidine blue staining (H). The arrows indicate that the non-specific binding of DAB products was used to align the photographs (G and H). Th: thalamus. Hip: hippocampus. Scale bar: 100 μm.

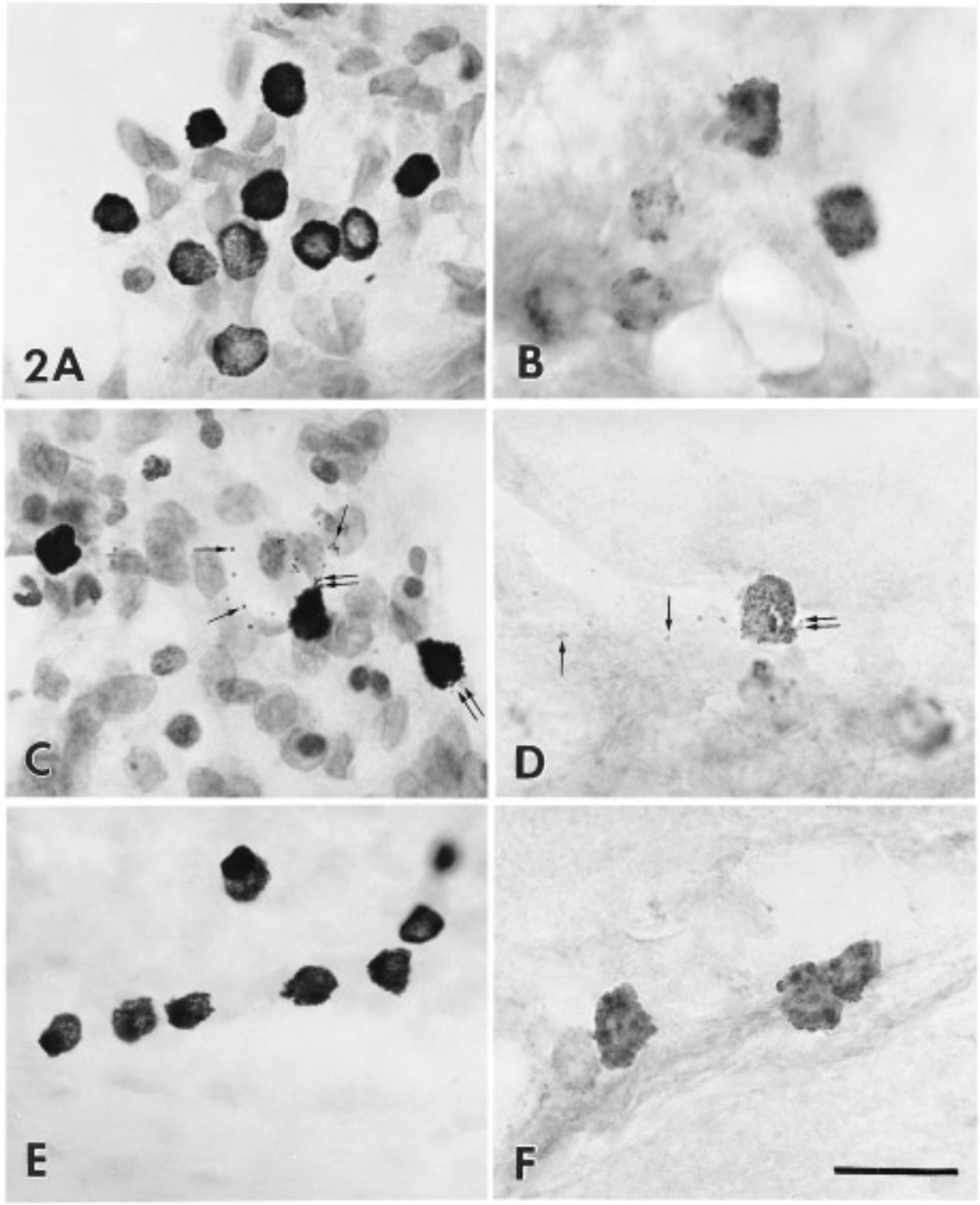

Fig. 2. Brain mast cells after C48/80 treatment. The left frames are stained with toluidine blue, and the right frames show DAB-labeled GnRH-like IR. In the left frames, intact mast cells from a control animal (A) have a clear cell boundary and contain abundant metachromatic cytoplasmic granules. Extruded granules (double arrows) are present in the extracellular space, and some are dispersed far from the cell body (arrows) 2 h after C48/80 treatment (C). The mast cells again resemble control cells 12 h after C48/80 treatment (E). GnRH-like IR is seen in mast cells of both the control (B) and C48/80-treated groups (D: 2 h, and F: 12 h after treatment). It should be noted that at 2 h after C48/80 treatment, the extruded (double arrows) and dispersed (arrows) granules also show GnRH-like IR (D). Scale bar: 20 μm.

were restored to their normal morphology (Fig. 2F) and showed GnRH-like IR distribution that was similar to that seen in the controls (Fig. 2B).

Mesenteric mast cells were evenly distributed. They displayed abundant granules and clear cell boundaries in the sham-injected control group (Fig. 3A). The mean diameter of the mesenteric mast cells was 15.91±2.31 μm. Mast cell degranulation was seen with numerous extruded granules both near and at a distance from the cell body (further than 30 μm) 15 min after C48/80 was administered intraperitoneally (Fig. 3C). The majority of granules were seen at a distance from the cell body, and toluidine blue staining intensity of the granules was significantly reduced 2 h after C48/80 treatment (Fig. 3E). Extracellular granules gradually disappeared within 4-9 h after C48/80 treatment. Mast cells were restored to their normal morphology 12 h after C48/80 treatment, with increased perinuclear granules and clear cell boundaries, but recovery was not complete, as granules were less abundant (Fig. 3G) than in control cells (Fig. 3A).

Immunohistochemical studies showed that GnRH-like IR was present in mesenteric mast cells of both sham-injected control and C48/80-treated animals. In the control group, GnRH-like IR was prominent in mast cells (Fig. 3B). GnRH-like IR was seen in both cytoplasmic and extruded granules 15 min after C48/80 treatment (Fig. 3D). Two h after C48/80 treatment, the extruded granules were located more than 30 μm from the cell body, and GnRH-like IR was weak (Fig. 3F). Mast cells had decreased in size (Fig. 3H) 12 h after C48/80 treatment, and GnRH-like IR appeared unchanged as compared with control mast cells (Fig. 3B).

Immunoelectron microscopic examination

In both sham-injected control and C48/80-treated animals, GnRH-like IR was seen in mast cell granules (Fig. 4A, B).

In the control group, the surface folds extended straight from the cell surface (length 0.2-0.9 μm). Numerous granules (diameter 0.2-0.7 μm) with GnRH-like IR were present at the cell periphery. Few GnRH-like immunoreactive granules appeared to be extruded from the cell bodies and were seen near the cell surfaces (Fig. 4A). Two h after C48/80 treatment, there were more GnRH-like immunoreactive granules located outside the cell body. In addition, profiles of long (0.7-2.0 μm) and curved surface folds were observed (Fig. 4B).

In the mesentery, mast cells were located in the connective tissue and were surrounded by abundant collagen fibrils. GnRH-like IR was seen in the majority of mesenteric mast cell granules, except for some perinuclear ones, but it was absent from the nucleus and surface cell membrane folds (Fig. 5A). Fifteen min after C48/80 treatment, numerous GnRH-like immunoreactive granules had been extruded into the extracellular space. The degranulated cells contained fewer perinuclear granules, most of which were not immunolabeled. Very few cell membrane folds were seen in the degranulated cell. At this time, some of the extruded granules were enclosed by the processes of connective tissue cells, around which numerous collagenous fibrils were identified (Fig. 5B), this having been completed by 2 h after C48/80 treatment (Fig. 5B, inset).

DISCUSSION

Specificity of GnRH-like IR in brain and mesenteric mast cells

It has been suggested that mast cells may bind antibodies in a non-specific manner (Simson et al. 1977, Spicer et al. 1994, Botchkarev et al. 1997). Gill and Rissman (1998) reported that both GnRH-specific and non-specific IRs were found in brain mast cells of the musk shrew. A series of experiments has proven that GnRH mRNA and GnRH peptides are found in another immune cell type, viz., lymphocytes, in the rat, pig, and human (Emanuele et al. 1990, Azad et al. 1991, Azad et al. 1993, Weesner et al. 1997, Chen et al. 1999). In the present study, we found that (1) no GnRH-like IR was seen in sections incubated in PBS without the primary antibody, (2) GnRH-like IR was markedly reduced in the presence of specific antibody preabsorbed with GnRH, and (3) only very weak staining was seen when using an irrelevant antibody. Therefore, we have confirmed that the immunostaining of GnRH-IR in brain and mesenteric mast cells in the present study is specific, and that there is also very weak non-specific binding.

GnRH-like IR in brain and mesenteric mast cells

Mast cells produce many mediators, including histamine, serotonin, cytokines, a variety of proteoglycans, protease, tryptase, neuropeptides, and nitric oxide (Silver et al. 1996). Previous studies have indicated that GnRH-like IR is expressed in

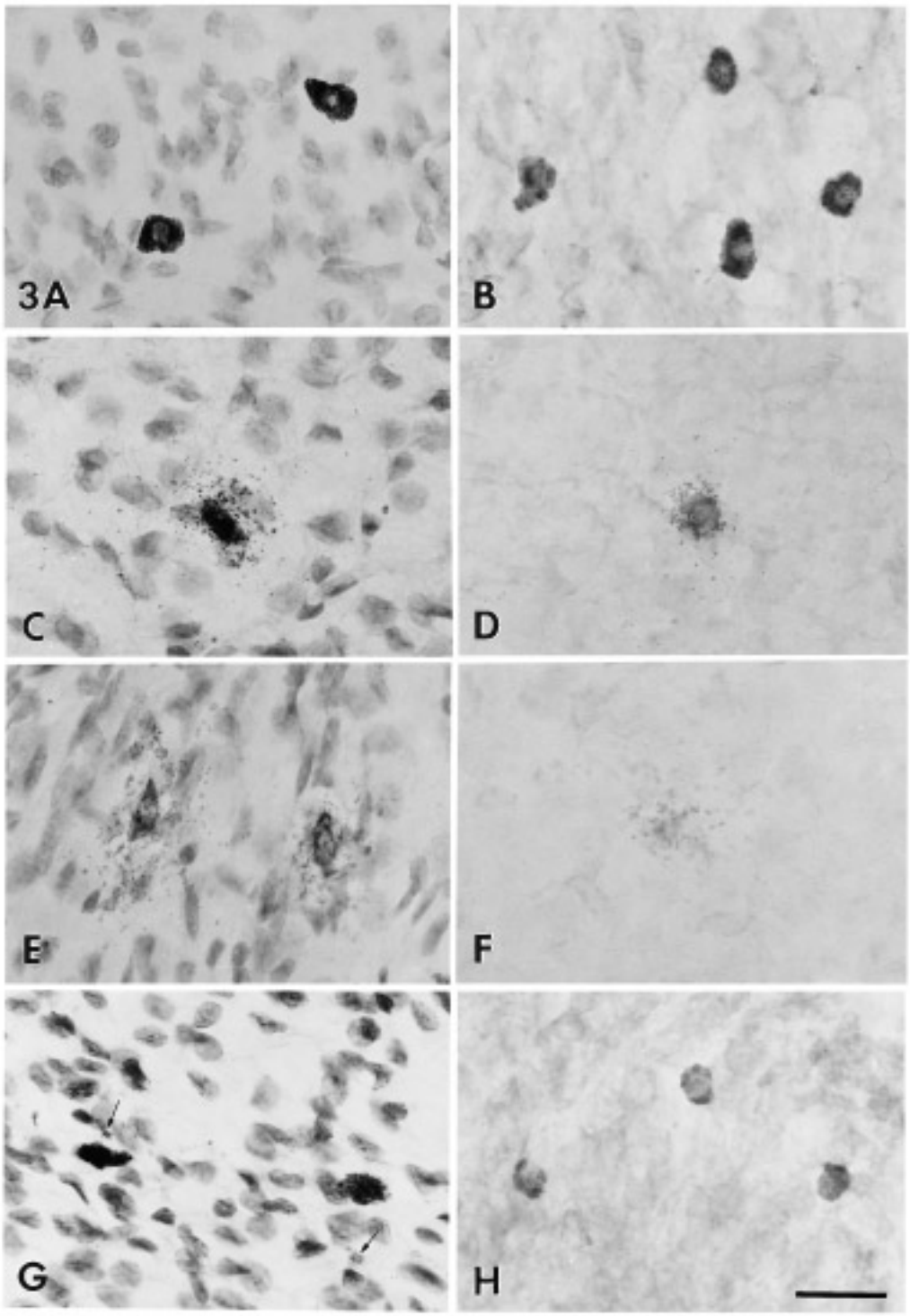

Fig. 3. Mesenteric mast cells after C48/80 treatment. The left frames are stained with toluidine blue, and the right frames show DAB-labeled GnRH-like IR. Mast cells with distinct cell boundaries and abundant cytoplasmic granules can be clearly seen in the sham-injected group (A). Extruded granules are found around the cell body 15 min after C48/80 treatment (C). Most of the extruded granules are dispersed far from the cell body, the nucleus is distinct and unstained, and the staining intensity of granules is reduced 2 h after C48/80 treatment (E). Mast cells are restored to their normal morphology, and far fewer extruded granules (arrows) can be seen in the vicinity of the mast cells 12 h after C48/80 treatment (G). The right frames show that GnRH-like IR is expressed in mast cells of sham-injected control animals (B) and in C48/80-treated animals 15 min (D), 2 h (F), and 12 h (H) after C48/80 treatment. The extruded granules show GnRH-like IR (D and F), but the intensity is reduced 2 h after C48/80 treatment (F). The GnRH-like immunoreactive material is seen in cells with the same morphology as in the controls 12 h after C48/80 treatment (H). Scale bar: 25 μm.

brain mast cells of the dove (Silver et al. 1992, Zhuang et al. 1993, Silverman et al. 1994), musk shrew (Gill and Rissman 1998), and mouse (Yang et al. 1999), suggesting an association between the neuroendocrine and immune systems in the brain. Mast cells are well known for their close appositions to the nervous system, such as to the enteric nerves of the intestine (Stead et al. 1987), vagus nerves of the mesentery in the rat (Rothschild et al. 1991), neurons in the dove medial habenula (Silver et al. 1996), and trigeminal sensory fibers in the rat dura mater (Dimitriadou et al. 1997). Electrical trigeminal stimulation promotes mast cell secretion and degranulation in the dura mater and tongue, and this activation of mast cells by neurogenic mechanisms appears to be important in the development of neurogenic inflammation (Dimitriadou et al. 1991). GnRH acts in the hypothalamic-pituitary system, and also acts as a peripheral modulator of reproductive functions, an immunomodulator in the thymus, and a neurotransmitter and/or neuromodulator in a variety of neural tissues (Marchetti et al. 1998). We found GnRH-like IR to be present in both brain and mesenteric mast cells. As far as we are aware, this is the 1st demonstration of the expression of GnRH-like IR in mesenteric mast cells. Thus the functional roles of GnRH in mast cells of the brain or mesentery and the relationship between GnRH-containing mast cells and their extracellular milieu need further investigation.

Degranulation of mast cells

It has been reported that degranulation of mast cells is rapid after in vitro treatments. In in vitro systems, rat mast cell degranulation occurs after 1 s to 20 min (Horsfield 1965) or 1-2 min following C48/80 application (Neilsen et al. 1981), 1-10 min after antigen induction (Anderson et al. 1973), and 5-30 s after exposure to bee venom or n-decylamine (Bloom et al. 1967). These studies revealed that mast cell degranulation is immediate in drug-supplemented culture. Bloom and Haegermark (1965) reported that the morphology of rat peritoneal mast cells treated with the lowest dose of C48/80 (0.1 µg/ml) in vitro, differed only slightly from controls, whereas in specimens treated with the highest dose (0.5 µg/ml), only a few intact mast cells were present. Thus they proposed that the extent of morphological changes of mast cells is directly related to the concentration of C48/80. In the present study, dramatic degranulation of mesenteric mast cells occurred as early as 15 min after C48/80 was injected intraperitoneally, suggesting that mesenteric mast cells were rapidly and directly affected by C48/80.

In a study of pericorneal mast cells, degranulation was seen 1 h after intraperitoneal injection of C48/80; this degranulating process persisted for

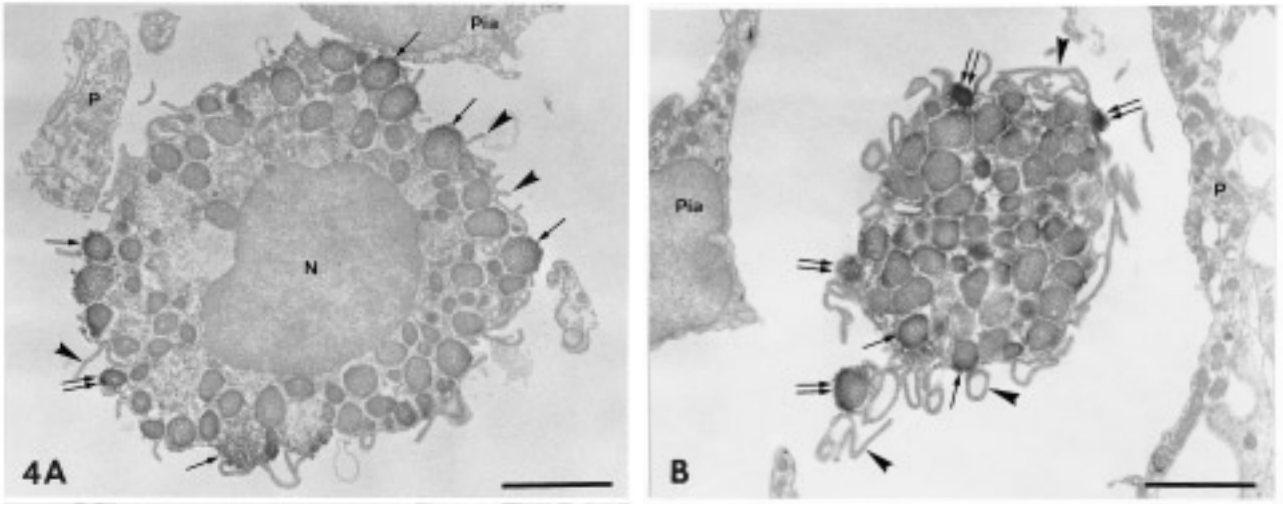

Fig. 4. Electron micrographs of GnRH-like immunoreactive brain mast cells in the velum interpositum. A: Mast cells from a sham-injected animal. Note that GnRH-like immunoreactive products are concentrated in some of the granules (arrows), which are mainly located at the cell periphery. An extruded granule (double arrow) can also be seen. No immunoreactive material is seen in the nucleus (N) or surface folds (arrowheads). Pia mater cells (Pia) and their processes (P) can be seen around the mast cell. B: Mast cells 2 h after C48/80 treatment. GnRH-like immunoreactive material can be seen in cytoplasmic (arrows) and extruded (double arrows) granules. The surface folds (arrowheads) of mast cells are longer and some are curved. Note that the pia mater cells (Pia) and their processes (P) are found around the mast cell. Scale bar: 2 µm.

6 h, and intact mast cells were shown 12 h after C48/80 injection (Garden 1965). In the present study, obvious degranulation of brain mast cells was seen 2 h after treatment, the degranulation persisted for several hours, and mast cells had been restored to a normal configuration 12 h after treatment. This suggests that brain mast cells undergo similar morphological changes to those of pericorneal mast cells in response to C48/80.

Release pattern of GnRH-IR granules

Pretreatment of rat peritoneal mast cells with various psychotropic agents results in the preferential release of serotonin, with no substantial release of histamine or massive degranulation (Theoharides et al. 1985). Becker et al. (1986) also demonstrated that cutaneous mast cells from atopic dogs could release histamine or other mediators without degranulation. This phenomenon is regarded as a "slow-release reaction" or "piecemeal degranulation" (Dvorak 1986). The morphologic expression of this process is the progressive loss of granular material, which is transported by vesicles to the cell surface (Dvorak 1986). In contrast, degranulation, in which the entire granule is extruded, is regarded as a "rapid-release reaction". In the present study, since GnRH-like immunoreactive granules were extruded to the extracellular space with a compact morphological appearance, we judged that the GnRH release of brain and mesenteric mast cells is a "rapid-release reaction" rather than a "slow-release reaction".

Mast cells after degranulation

Greenberg and Burnstock (1983) demonstrated that transgranulation occurs between mast cells and fibroblasts with mast cells apparently transferring their granules to the cytoplasm of fibroblasts or to the mesothelium. Norrby and Enestrom (1984) reported that mast cell granules are internalized in fibroblasts 1-3 h after C48/80 injection. Takayama et al. (1994) reported that the extruded granules might be degraded by the extracellular milieu, as the initial compact morphological appearance of the discharged granules is gradually lost, and the granule contents are discharged. Two h after C48/80 treatment, numerous GnRH-like immunoreactive granules were found in the extracellular milieu of mast cells, and some of the extruded GnRH-like immunoreactive granules were encircled by connective tissue cell processes (Fig. 5B, inset). This indicates that transgranulation from mast cells to other cells can occur in C48/80-treated animals. These results may also suggest that the extruded GnRH-like immunoreactive granules were later destroyed by the extracellular milieu, and that GnRH had diffused and been transported to target cells through blood circula-

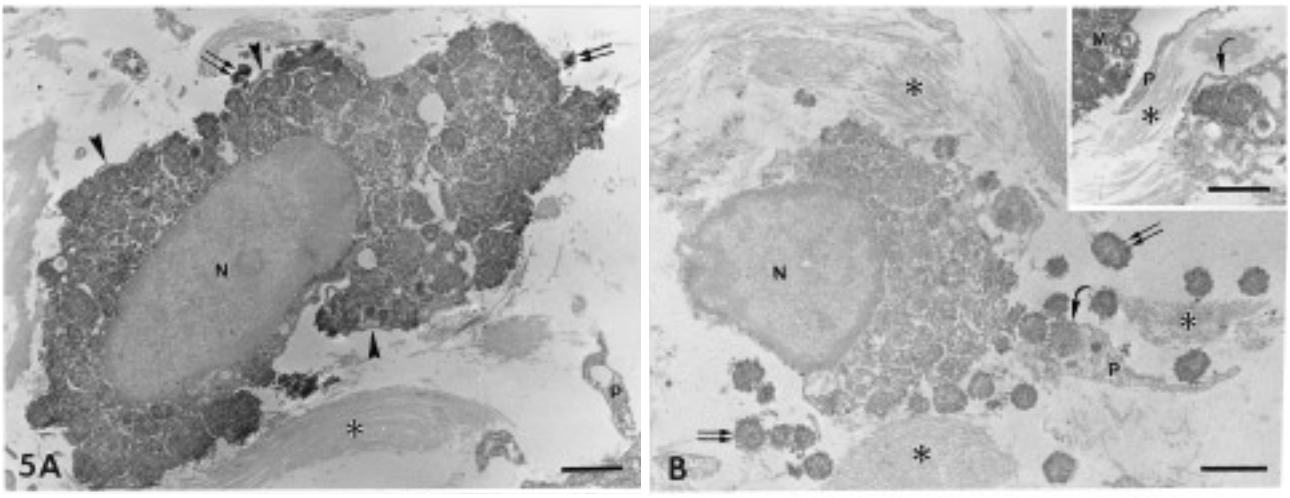

Fig. 5. Electron micrographs of GnRH-like immunoreactive mesenteric mast cells. A: Mast cells from a sham-injected animal. GnRH-like immunoreactive products are present in the majority of cytoplasmic granules, but not in the nucleus (N), or surface folds (arrowheads). Some extruded granules (double arrows) are located near the cell body. Collagen fibrils (*) and connective tissue cell processes (P) are distributed around the mast cell. B: Mast cells 15 min after C48/80 treatment. Many extracellular granules (double arrows) containing immunoreactive material can be clearly seen. A connective tissue cell process (P) with many small vesicles wraps around an extruded granule (curved arrow). Inset: Two h after C48/80 treatment. The extruded granule is encircled by the connective tissue cell process (curved arrow). Part of the mast cell (M) and connective tissue cell process (P) can also be seen. N: nucleus. *: collagen fibrils. Scale bar: 2 μm.

tion.

Previous reports have indicated that the recovery of degranulated rat peritoneal mast cells is not complete 48 h after C48/80 treatment in vitro (Bytzer et al. 1981). Chen and He (1990) reported that mast cells degranulate and regranulate within 24 h under physiological conditions. In the present study, mast cells were almost restored to their normal morphology (i.e., a normal density of granules and a clear cell boundary of mast cells), 12 h after treatment, indicating that mast cells are able to recover themselves after being affected by C48/80. Slutsky et al. (1987) suggested that the process of recovery in degranulated rat peritoneal mast cells after chemical stimulation is dependent upon cellular metabolism and involves the interaction of Ca, calmodulin, and microfilaments. However, the details of this interaction and the mechanism responsible for recovery remain to be elucidated.

Zhuang et al. (1996) reported that local degranulation of dove brain mast cells alters the blood-brain barrier permeability 2 h after C48/80 intramuscular administration. Norbby et al. (1976) reported that in rat mesentery, the proliferation of normal mesenchymal cells adjacent to activated and degranulated mast cells had significantly increased 24 to 32 h after a single intraperitoneal injection of C48/80. These and our present results indicate that degranulation of mast cells induced by C48/80 plays a pathophysiological role in regulating the extracellular milieu. Because the abundance of mast cells in the gerbil makes it easy and feasible to study the effects of mast cell actions under various experimental conditions in vivo, we recommend the gerbil as a good animal model for the research of the interaction between mast cells and the extracellular milieu.

In summary, the present study shows that in the gerbil, both brain and mesenteric mast cells express GnRH-like IR as determined by immunohistochemistry and immunoelectron microscopy, and that the GnRH-immunoreactive granules are liberated by a rapid-release reaction after C48/80 administration. This study suggests that the gerbil is a good animal model for mast cells studies in vivo and extends our knowledge of the dynamic changes of mast cell granule release that occur after C48/80 treatment.

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化合物 48/80 誘發的沙鼠腦及腸繫膜內具 GnRH 免疫反應之肥大細胞的去顆粒化

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肥大細胞可經由去顆粒化（degranulation）而影響其細胞外環境。近來的研究結果顯示，鴿子及哺乳類的腦肥大細胞可表現促性腺激素釋放激素（Gonadotropin-releasing hormone, GnRH）的免疫反應。本研究的目的在探討 GnRH 於腦及腸繫膜肥大細胞的表現，以及投予化合物 48/80 後其去顆粒化情形。本研究採用沙鼠為實驗動物，因沙鼠的腦及腸繫膜內具有大量肥大細胞。本研究之結果顯示：腦及腸系膜的肥大細胞，不論是對照組或投藥組皆可表現 GnRH 的免疫反應。腸繫膜肥大細胞於腹腔內投藥後有非常劇烈的去顆粒化反應。電子顯微鏡觀察結果顯示，沙鼠之腦及腸繫膜肥大細胞所釋放具有 GnRH 免疫反應的顆粒都相當緻密，且釋放顆粒後的肥大細胞可於投藥後十二小時復原。由上述結果可得以下結論：（一）腦及腸繫膜肥大細胞皆可表現 GnRH 的免疫反應，以及（二）GnRH 是以快速的釋放反應經由肥大細胞釋放出來。

關鍵詞：去顆粒化，肥大細胞，促性腺激素釋放激素，腦，腸繫膜。

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