The Role of Vagal Reflex in Mechanism of Secretagogic Action of Bromhexine

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Abstract—The in vivo effect of bromhexine on secretory activities of tracheal submucosal glands was investigated with a histological/histochemical technique with reference to a role of the vagal reflex. When bromhexine was given at 5, 10 or 20 mg/kg into the stomach of anesthetized dogs, the ratio of acinar inner diameter of the submucosal gland to wall thickness (AIWR) markedly increased in a biphasic manner; the early transient increase was seen 0.5 hr after administration, and the second prolonged increase occurred during 2 to 6 hr after administration. The early stimulant phase was almost abolished by atropine, 1 mg/kg i.v., or bilateral cervical vagotomy, whereas the second stimulant phase was not affected by these treatments. Emetine also induced a similar early increase in AIWR at 0.5 hr after administration, the change also being abolished by atropine or surgical vagotomy in this case. The number of submucosal glandular cells which stained blue and purple with a combination of alcian blue at pH 2.5 and periodic acid-Schiff was decreased by bromhexine, but the cell number which stained red was markedly increased. These histochemical changes in glandular cells were not influenced by treatment with atropine or surgical vagotomy. In the present study, it was found that bromhexine exerts both a secretagogic action on submucosal glands and a mucolytic action toward acid glycoproteins inside the cells in vivo. Also, the secretagogic action of bromhexine occurs biphasically; the first phase results from the vagal reflex probably through a gastrointestinal irritation, and the second phase results from a direct action on the glands.

Excessive production of viscous mucus in the airway is a characteristic feature in chronic obstructive pulmonary diseases. The overproduced viscous mucus causes an inhibition of the function of the airway clearing system and thereby becomes the cause of obstruction and infection of the respiratory tract. In the treatment of these symptoms, expectorants have been widely used to prompt the expelling of the mucus plug.

It is known that bromhexine, N-(2-amino-3,5-dibromobenzyl)-N-cyclohexyl-methylamine, is an efficient expectorant possessing both secretagogic action of respiratory tract fluid (RTF) (1-4) and mucolytic action on respiratory mucus (1, 5, 6). The mechanism for the expectorant action of bromhexine, however, has not been well understood. It was found that the secretagogic action of bromhexine results from a selective promotion of the secretory activity of submucosal glands in the lamina propria mucosae of the respiratory tract and that the mucolytic action of the agent results from a dissolution of acid glycoprotein (AGP), a chief viscous factor (7, 8), contained in mucus granules of both goblet cells in the epithelium and submucosal glandular cells (9-11). In a previous paper, we reported an in vitro result that the
The mucolytic action of bromhexine is, at least in part, derived from the effect of this agent to liberate lysosomal enzymes into the cytoplasm inside secretory cells (11). The lysosomal enzymes would enzymatically dissolve the high molecular AGP of mucus.

In the present study, we investigated the in vivo expectorant action of bromhexine using the histological/histochemical technique, with reference to the role of the vagal reflex.

**Materials and Methods**

**Animals:** Male adult mongrel dogs weighing between 9 and 14 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The animals were fasted for 17 hr prior to drug administration.

**Drug administration:** Bromhexine or emetine to be tested was administered directly into the stomach through a gastric tube in a volume of 2 ml/kg. In some experiments, pretreatment with atropine (1 mg/kg i.v.) or bilateral cervical vagotomy was made, about 5 min prior to bromhexine or emetine administration. As a control, distilled water (2 ml/kg, at room temperature) was given intragastrically instead of bromhexine or emetine. The drugs used were bromhexine hydrochloride (Nippon Boehringer Ingelheim Co.), emetine hydrochloride (Tokyo Kasei) and atropine sulfate (Tokyo Kasei). Bromhexine and emetine were dissolved or diluted with distilled water, and atropine was dissolved with 0.9% NaCl solution. Doses of all the drugs were expressed in terms of their bases.

**Tissue preparation:** Tissue biopsy of a part of the trachea was carried out as follows: The tracheal tissue (about 0.7 cm in length x 1 cm in width) was excised at the 8th tracheal ring from the larynx after a midincision. Then, the tracheal tissues of the same size at the 6th and 4th tracheal rings from the larynx were excised in turn. Two animal groups were made. In one group, biopsies were carried out 0.5 hr before and 1 and 4 hr after administration of expectorant. In another group, biopsies were made 0.5, 2 and 6 hr after administration of expectorant. After excising the tracheal tissue, the remaining cut edge was cauterized with a high frequency knife (Takahashi, Midget Super) to prevent bleeding. The excised tracheal tissues were fixed with 10% neutral formalin for 7 days at room temperature and cut into small pieces (about 5 mm x 5 mm). The pieces were then washed for 15 hr in running water. After washing, the tissues were dehydrated with ethyl alcohol, infiltrated with paraffin wax, and sectioned at 4 µm thickness. The tissue slices were stained with a combination of alcian blue (AB) at pH 2.5 and periodic acid-Schiff (PAS) (designated as AB (pH 2.5)/PAS) (12).

**Evaluation of histological and histochemical changes in tracheal submucosal glands:** Four to six dogs were used to assess the effect of each drug. Fifty slices were prepared from a tracheal tissue, and 10 out of the 50 slices were chosen at random to be stained and photographed at 150-fold magnification.

The analytical method for photomicrographs of submucosal glands in the tracheal tissues were described previously (9–11, 13–18). In this study, the ratio of acinar inner diameter of submucosal gland to the tracheal wall thickness (A'WR) was used to represent the histological parameters. The wall thickness means the tissue thickness between the basement membrane of the epithelium and the inner side of the cartilage. As a histochemical parameter, the color (stain index) of the glandular cell in the AB (pH 2.5)/PAS procedure was used.

**Results**

**Time course of bromhexine-induced changes in tracheal submucosal glands:** Changes in A'WR of submucosal glands following bromhexine treatment are shown in Fig. 1. The precontrol A'WR level was 0.050±0.002 (mean±S.E., N=19). In the control group, A'WR remained much the same throughout a 6-hr observation period. On the other hand, when bromhexine was given at 10 or 20 mg/kg, A'WR significantly increased biphasically; one peak was observed at 0.5 hr after drug administration, and the other A'WR increase was after 2 to 6 hr. In the 5 mg/kg group, a similar pattern was obtained, although the changes were small.
In Fig. 2 are shown changes in the stain index of submucosal glandular cells with the AB (pH 2.5)/PAS procedure after bromhexine treatment. In the precontrol tissue, the percentages of glandular cells that stained blue (stain index B), purple (stain index P) and red (stain index R) were 62.1%, 26.6% and 11.3%, respectively (total number of glandular cells evaluated being 321). The stain index of glandular cells remained almost unchanged throughout a 6-hr period in the control group. On the other hand, bromhexine at 5, 10 or 20 mg/kg caused a decrease in the number of glandular cells showing a stain index B&P and caused a marked increase in the number of cells showing a stain index R. These histochemical changes following bromhexine treatment lasted for at least 6 hr.

Effects of atropine or cervical vagotomy on bromhexine-induced changes in tracheal submucosal glands: In contrast to bromhexine, emetine (1 mg/kg) caused a significant increase in AIWR at 0.5 hr after administration, followed by a significant decrease. The AIWR then recovered to the control level after 4 or 6 hr (Fig. 3).

After pretreatment with atropine at 1 mg/kg i.v. and bilateral cervical vagotomy, the precontrol AIWR levels were 0.045±0.003 (N=15) and 0.047±0.004 (N=16), respectively. The transient increase in AIWR that

![Fig. 1. Time course of AlWR changes in tracheal submucosal glands when bromhexine was given intragastrically to anesthetized dogs. Each value represents the mean±S.E. of 4–5 experiments (Each experimental datum is the mean of results determined from 10 slices). AlWR: the ratio of acinar inner diameter of submucosal gland to wall thickness. Significant at *P<0.05, **P<0.01 and ***P<0.001 as compared to the control value.](image)

![Fig. 2. Time course of stain index changes in tracheal submucosal glandular cells when bromhexine was given intragastrically to anesthetized dogs. Each circle represents the percent of cells stained blue (stain index B), purple (stain index P), or red (stain index R) in the combination procedure of alcian blue at pH 2.5 and periodic acid-Schiff (AB (pH 2.5)/PAS). The numbers of glandular cells evaluated were 321 (precontrol), 98–211 (control), 88–148 (bromhexine, 5 mg/kg), 185–247 (10 mg/kg) and 168–224 (20 mg/kg).](image)
was seen at 0.5 hr following bromhexine (10 mg/kg) treatment was almost abolished by atropine or surgical vagotomy, whereas the 2nd phase increase in AIWR was not influenced by these vagal blockades. The emetine-induced transient increase and after-decrease in AIWR were prevented by treatment with atropine or surgical vagotomy (Figs. 4 and 5).

Bromhexine (10 mg/kg)-induced histochemical changes in submucosal glandular cells were not affected throughout a 6-hr period by treatment with atropine or surgical vagotomy.

Emetine (1 mg/kg) caused almost no change in histochemical parameters of submucosal glandular cells throughout a 6-hr period.

**Discussion**

In 1963, Engelhorn and Puschmann (1) introduced bromhexine as an expectorant having both secretagogic action on RTF and mucolytic action on respiratory mucus. Afterwards, these actions have been confirmed in many laboratory and clinical studies (2–6). The detailed mechanism of actions of bromhexine, however, has not been known until recently. In 1981, in an in vitro experiment using isolated canine trachea, it was found that bromhexine stimulates the secretory activity of the submucosal gland in a selective manner, and additionally, it dissolves AGP in mucus granules of both goblet and submucosal glandular cells (9, 10). Furthermore, it was recently reported that bromhexine liberates...
lysosomal enzymes into the cytoplasm, and the enzymes liberated would then exert a mucolytic action on AGP contained in mucus granules of airway secreatory cells (11).

In the present study where bromhexine was administered into the stomach, AIWR markedly increased after 0.5 hr, followed by a decrease after 1 hr, and it significantly increased again after 2 to 6 hr. These histological findings suggest that the in vivo secretagogic action of bromhexine on submucosal glands occurs biphasically: the first being a transient phase and the second, a prolonged phase.

Engelhorn and Püschmann (1) reported that the increased volume of RTF in rabbits after administration of bromhexine in a dose of 2 mg/kg into the stomach was not reduced by 1 mg/kg atropine sufficiently to block cholinergic effects. Also, Boyd and Sheppard (2) showed that the increase in RTF output produced by bromhexine (5 mg/kg into the stomach) in rabbits was not affected by atropine in doses of 20 and 40 mg/kg s.c., although atropine in toxic doses (60 and 80 mg/kg s.c.) almost abolished the response. They concluded that bromhexine would increase RTF output through a direct action on airway tissue and not through a vagal reflex or any cholinergic nervous mechanisms.

In the present study, emetine, presumably a reflexive secretagogue (19, 20), produced a marked increase in AIWR at 0.5 hr after administration into the stomach, followed by a decrease. The emetine-induced changes in AIWR were inhibited by atropine or bilateral cervical vagotomy. This result suggests that the secretagogic action of emetine on submucosal glands may be chiefly caused by the vagal reflex through a gastrointestinal irritation. The decrease in AIWR seen after 1 to 2 hr might occur as a rebound from the preceding increase in AIWR. Similarly, the bromhexine-induced early increase in AIWR seen after 0.5 hr was also inhibited by atropine in a dose usually used for cholinergic blockade or by surgical vagotomy, indicating that in this secretagogic phase, the vagal reflex from the gastric mucosa may be involved. The second secretagogic phase seen during 2 to 6 hr after bromhexine treatment was not, however, influenced by either cholinergic blockade. These findings strongly suggest that bromhexine works through both mechanisms: a vagal reflex from the gastric mucosa and a direct stimulant action on submucosal glands. In the studies of Engelhorn and Püschmann (1) and Boyd and Sheppard (2) described above, they might have been unable to detect the early secretagogic action of bromhexine through the vagal reflex because in their experiments, RTF volumes were measured at every 2 hr after administration.

After bromhexine treatment, the number of submucosal glandular cells showing a stain index B &P was markedly decreased, while the number of cells showing a stain index R was increased. These histochemical changes appeared strongly 1 to 6 hr after drug administration. These findings suggest that bromhexine may dissolve high molecular weight AGP in granules of glandular cells through a mucolytic action because AB colors blue only when a single dye molecule combines with several polyanion sites on one AGP molecule (21, 22). Furthermore, the time course of the histochemical changes suggests that there is a lag time before onset of the strong mucolytic action of bromhexine. The reason why it takes time for the agent to produce intense mucolysis in contrast to its secretagogic action seems to be that the mucolytic action of bromhexine is induced enzymatically through a liberation of lysosomal enzymes in the tracheal tissue. That is, the mucolytic action is considered to be elicited through a direct action on the tracheal tissue, so a sufficient plasma concentration has to be attained after intragastric administration. This may be supported by the finding that the histochemical changes were not influenced by cholinergic blockade. The degrees of histological and histochemical changes observed in this in vivo study using 10–20 mg/kg of bromhexine roughly resembled those observed in the in vitro study using 10^{-6}–10^{-5} M/l of bromhexine.

In conclusion, the secretagogic action of bromhexine on submucosal glands occurs biphasically: the first phase results from the vagal reflex probably through a gastrointestinal irritation, and the second phase results from a direct action on the glands.
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