Actual pathway of intraductally injected substances in the rat submandibular gland

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Introduction

Foreign substances, for example, radiopaque substances and various pharmacological drugs, are routinely injected retrogradely into salivary glands via their ducts in various clinical and experimental procedures. Such procedures more or less cause an increase in intraluminal pressure, and the gland may thus be damaged when exposed to such pressure. But little is known about either the glandular response to intraluminal pressure or the movement, distribution and fate of the injected substances within the gland, following such ductal injections.

We recently found\(^1\) that an increase in intraluminal pressure increases the concentration of glucose in submandibular saliva and suggested that this effect was a result of damage to the sealing (or barrier) mechanism of intercellular junctions, perhaps at the molecular level, but mechanical disruption.

In this study, we examined the recovery from the damage induced by intraductal injection in the gland, and the movement of intraductally injected solutions. Horseradish peroxidase (HRP), a water soluble protein with a molecular weight of approximately 40,000 and a diameter 3.5–4.0 nm\(^2\), was chosen as a suitable marker for this study because it can be traced in the gland histochromically at both light and electron microscopic levels.

Materials and Methods

Male Wistar rats weighing 250–300 g were used. The animals were anesthetized with sodium pentobarbital injected intraperitoneally (65 mg/kg). The polyethylene tubing (Clay Adams, PE10), which was used for intraductal injection, was inserted into the oral opening of the submandibular duct and was fixed in place with adhesive.

The gland was injected manually with solutions as uniformly as possible over a 20-second period using a microsyringe attached to the tubing, with a pressure transducer connected between the syringe and the tubing. The time from beginning of the injection until pressure release was 3 minutes. The injected fluids were either isotonic saline or

![Fig. 1 Relationship between injection volume and intraductal pressure. The inset shows typical intraductal pressure changes when 20µl of normal saline is injected and the pressure maintained for 3 minutes. The initial steep rise in pressure is produced artificially by the intraductal injection of the solution. The data are presented as mean±SE of 5 experiments.](image-url)
Fig. 2 Light micrographs of rat submandibular glands after injection of various volumes of normal saline; toluidine blue staining. 1) Control; 2) Injection of 30 μl: Some vacuolization is found in the duct cells. A slight increase in the amount of extracellular space is noted, 3) Injection of 40 μl: Severe vacuolization is found in the duct cells and oedematous damage has also occurred; 4) Injection of 60 μl: Vacuolization is very severe. Some duct cells are broken with oedematous damage.
horseradish peroxidase (Sigma, HRP type II) dissolved in isotonic saline at a concentration of 50 mg/ml. The pressure was subsequently released after the injection, and the gland was then removed from the animal.

Tissues were fixed by immersion, using a glutaraldehyde mixture, and sections of the tissues were treated in the usual way. Peroxidase activity was demonstrated by the method of Graham, R. C. and Karnovsky, M. J. (1966)\(^3\), and specimens were examined at both the light and electron microscopic levels.

**Results and Discussion**

**Intraductal pressure**

When 10–60 µl of normal saline was injected into the duct and the pressure maintained for 3 minutes, the intraductal pressure increased as the injected volume was increased, gradually reaching a plateau value after more than 30 µl was given (Fig. 1).

**Normal saline injections**

Some sialographic studies have been carried out to assess the glandular damage due to exposure to intraductal pressure. Dawes, C. et al. (1978)\(^4\) showed in their sialographic study on human parotid glands that a pressure of 200 mmHg revealed no evidence of permanent duct damage. Siegel, I. A. (1976)\(^5\), who maintained a pressure of 65 mmHg for 15 minutes in dog parotid glands, also found no histological evidence of damage to the glands.

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**Fig. 3** Light micrographs of rat submandibular glands 24 hours and 3 days after injection of 40 µl of isotonic saline. 1) 24 hours after injection: Vacuolization is not found. The tissue has almost recovered from the oedematous damage. 2) Three days after injection: The gland has completely recovered and appears normal.
Fig. 4 Electron micrographs of rat submandibular glands after being injected with various volumes of HRP solution injections. 1) 10 µl: The HRP reaction products are found in the lumen (L), intercellular space (IS) and basal membrane (BM) of the duct cells. Cellular damage has not occurred; 2) 20 µl: The HRP reaction product is found in intercellular space (IS) and basal membrane (BM). Some vacuoles (V) are found; 3) 40 µl: Disorder of luminal membrane of the duct cells are observed (*), but the luminal cell membrane is not broken. Some artifacts (art) are seen; 4) 40 µl: No HRP reaction product is found in the acinar lumen but was found in the basal intercellular space at the tight junction (†).
In our study, no histological changes were detected by the intraductal injection of less than 20 μl of isotonic saline; however, oedematous damage or vacuolization of the duct portion was induced by the intraductal injection of 40 μl of isotonic saline (Fig. 2). Similar ductal alteration, known as "ballooning disruption", has been observed by Emmelin, N. et al. (1977)⁶ in the dog submandibular gland. When more than 60 μl of normal saline was injected, cellular breakdown was observed in some duct portions. The tissue had recovered from most oedematous damage or vacuolization within 24 hours and completely within 72 hours (Fig. 3).

**HRP injections**

When 20 μl of HRP solution was injected, the reaction product of HRP was present in the interstices of the gland, ductal lumen and in the intercellular spaces between duct cells; but no HRP reaction product was seen in the acinar lumen. The cell membranes were not observed to have been damaged (Fig. 4).

The results indicate that HRP reaches the interstices of the gland mainly by penetration between adjacent duct cells. On the other hand, Garrett, J. R. and Parsons, P. A. (1976)⁷ showed that intraductally injected HRP reaches the interstices of the rabbit submandibular gland principally by penetration between acinar cells. Similar was observed in dog submandibular gland⁸.

Perhaps, most of the saliva that pre-existed in the lumen was displaced into the interstices via the intercellular junctions of both ductal and acinar cells.

The present study shows that when only a small volume of fluid is injected intraductally into the salivary gland, some of this fluid passes into the extracellular spaces of the gland via the intercellular junctions of the duct cells, even if no cellular breakdown occurs. This is schematically shown in Fig. 5.

We suggest that the intraductal injection of various drugs for experimental purposes affects not only the luminal site of the gland cells but also the basal site as well.

**References**


7) Garrett, J. R. and Parsons, P. A.: Movement of horseradish peroxidase in rabbit submandibular glands after ductal injection. Histo-