Test on Salivary Glands

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Contents

Measurement of Salivation

Purpose and Rationale

Symptoms of several human diseases are manifested as increased salivation (e.g., Parkinson's disease) or decreased salivation (e.g., xerosis). Studies to find and to evaluate sialagogues, such as substance P and its synthetic derivatives, as well as to search for salivation inhibitors are necessary. Saliva excretion is greatly influenced by anesthetics. Wagner et al. (1991) proposed a simple method to study saliva secretion in conscious rats and to evaluate sialagogues and sialagogue antagonists.

Procedure

Fed, male Sprague–Dawley rats (200–300 g) are weighed and distributed randomly into groups of six animals. Conscious rats are injected i.v., via the lateral tail vein, with either the vehicle or the sialagogue, e.g., substance P (0.3–3 μg/kg in 1 ml saline/kg). The rat's oral cavity is swabbed immediately after i.v. injection by placing and holding a pre-weighed, absorbent foam cube (e.g., 5/16″, Texwipe Company, Upper Saddle River, NJ) sublingually for 10 s using a triceps foam pencil (Texwipe Company, Upper Saddle River, NJ). Conscious rats are restrained during the 10 s collection period by gently holding the animal and opening the mouth using a plastic-coated snare,

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which is looped around the maxillary incisors and drawn back over the animal's head and the hand holding the rat, drawn around in front of the rat, and looped around the mandibular incisors. Gentle pressure on the snare opens the rat's mouth allowing the placement of the absorbent cube. Foam cubes are reweighed immediately after use. The difference between the initial weight of the cube and the weight of the cube after use represents saliva secreted.

Evaluation

Data are analyzed with Dunnett's *t*-test that compares several treated groups with a control group. Regression analysis is used to determine dose response and relative potency.

Modifications of the Method

Martinez et al. (1978, 1981) inserted appropriate plastic cannulae into the main excretory ducts of the two submandibular glands in **rats**.

Giuliani et al. (1988) studied the relative contributions of various neurokinin receptors (NK-1, NK-2, NK-3) to the sialogogic response after i.v. application in urethane-anesthetized rats.

Direct cannulation of the glandular duct with polyethylene tubing was performed by Bodner et al. (1983) and Kohn et al. (1992).

Bianciotti et al. (1994) cannulated the ducts of both the submaxillary and parotid glands in male Wistar rats anesthetized with 10 % ethyl urethane. No basal flow of saliva was observed from either gland; however, dose–response curves could be established after intravenous injection of sialogogic agents, such as methacholine (0.3–10.0 μg/kg), norepinephrine (3–60 μg/kg), isoproterenol (1–30 μg/kg), methoxamine (30–300 μg/kg), and substance P (0.3–10.0 μg/kg). Atrial natriuretic factor enhanced the salivary response to methacholine, methoxamine, and substance P.

Lohinai et al. (1997) determined salivary amylase secretion in conscious rats. Under ether anesthesia a catheter was introduced into the esophagus for salivary juice collection, and a cannula was inserted into the jugular vein for infusions. After postanesthesia recovery, submaximal carbachol infusion was given as a background to obtain steady secretion because of the low basal secretory rate. After application of drugs, volume and amylase were determined in saliva samples collected for 30 min.

Iwabuchi et al. (1994) studied salivary secretion after administration of a muscarinic agonist in MRL/lpr **mice**. Saliva was collected from the floor of the mouth of anesthetized rats with a capillary micropipette every 5 min for 60 min.

A method for the quantitative comparison of atropine substitutes on the salivary secretion of the **cat** has been published by Bülbring and Dawes (1945). Cats anesthetized with pentobarbitone were used. A cannula is tied into Wharton's duct and attached to a bottle containing tap water. The tap water, displaced by the saliva, passes out of the bottle through a tube which actuates a drop timer.

Ekström et al. (1994) used morphometric analyses to study the parotid acinar degranulation in cats after stimulation of the parasympathetic auriculotemporal nerve.

Izumi and Karita (1994, 1995a, b) investigated the secretory and vasodilator effects of nerve stimulation in the submandibular gland of cats. Cats of either sex were anesthetized with ketamine and a mixture of chloralose and urethane, paralyzed by intravenous injection of pancuronium bromide, and artificially ventilated. Blood flow changes in the submaxillary glands and lips of the cats were measured using a laser Doppler flowmeter. The duct of the submandibular gland was cannulated with a polyethylene cannula inserted distal to the intersection between the chorda lingual nerve and the duct. The amount of saliva secreted in response to nerve stimulation was determined gravimetrically by collecting the saliva in pre-weighed tubes.

Boldyreff (1925) described the preparation of salivary fistulae in the **dog**.

For preparing a **parotid fistula**, a fine sound is introduced through the orifice of the parotid duct, which is found opposite to the largest upper molar tooth, to the depth of 6–8 cm. Around the orifice

and at a distance of about 0.5 cm from it, four sutures are laid on the mucosa at equal distances one from the other. After this, a round piece of mucosae, about 1 cm in diameter around the orifice, with the sutures at the edge of this piece, is cut out with small sharp scissors. The duct is then separated from surrounding tissues about 2 cm from the orifice in the direction of its length. Then an opening is made through the cheek into the mouth (from the point half way on the vertical line from the front or the back corner of the eye to the mouth) to the base of the prepared duct. The orifice of the duct is now led outside by pulling out with the forceps. Four sutures on the piece of mucosa are made around it. The piece of mucosa is sutured carefully to the skin with knot sutures. The wound inside the mouth is closed with a continuous suture. The piece of mucosa must be covered daily with Vaseline to prevent drying. Sutures must be taken out slowly, beginning 3 days after operation. For the first 10 days after operation, it is necessary to produce on the dog an intensive salivary secretion, twice a day, by introducing into the mouth of the animal dry bread or meat powder or 0.5 % solution of hydrochloric acid. Saliva is collected into graduated test tubes.

In a similar way, one can produce a **fistula of the submaxillary or sublingual glands**, usually a common fistula for both glands, because their ducts have a common orifice.

Ogawa et al. (2003) developed a model of chronic parotitis in rats by a direct injection of complete Freund's adjuvant into the unilateral parotid gland via the parotid duct without skin incision.

Lambert et al. (1994) **cultured acinar cells** from lacrimal and submandibular glands as well as epithelial cells from rat small intestine in supplemented, serum-free media and measured the secretory components after treatment with various agents by radioimmunoassay.

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