The effect of prolonged treatment with amitriptyline on the secretory activity of rat salivary glands evoked by parasympathetic nerve stimulation and isoprenaline administration has been studied. Low doses of amitriptyline (10 mg/kg per day for 2 or 4 weeks), did not significantly affect salivary flow evoked by either parasympathetic nerve or isoprenaline stimulation. Higher doses of amitriptyline (50 mg/kg/day for 2 or 4 weeks) however, markedly decreased parasympathetic-evoked salivary secretion (flow and volume) from both parotid and submandibular glands, while isoprenaline-evoked secretions were unaffected. Sodium, potassium, and calcium concentrations of nerve-elicited or isoprenaline-evoked saliva were not significantly altered by amitriptyline treatment. Protein concentration and amylase activity of nerve-elicited parotid saliva were, however, greatly increased by chronic amitriptyline administration. Possible mechanisms for drug-induced increase in nerve-elicited salivary protein concentration include changes in cholinergic receptor binding, release of neuropeptides and variations in phosphatidylinositol turnover, which need further study.

Key words: Amitriptyline, autonomic stimulation, rat parotid, submandibular saliva

Introduction

Although tricyclic drugs such as amitriptyline have been widely used in the treatment of depression, it is still unclear how these drugs exert their therapeutic effects. However, one of the major side effects of these drugs is marked hyposecretion from the salivary glands as a result of anticholinergic activity. We recently reported that a single dose of amitriptyline (0.1–1 mg/kg, i.v.) had a strong anticholinergic-like effect on rat salivary glands concerning salivary flow. The calcium concentration of parasympathetically stimulated submandibular saliva was increased, but that of similarly evoked parotid saliva was not affected.

The present study was undertaken to investigate the effects of prolonged (2–4 weeks) administration of amitriptyline on the flow rate, sodium, potassium, calcium, and protein concentrations, and amylase activity of both parasympathetically and sympathetically stimulated salivas from the parotid and submandibular glands of the rat.

Methods

Animals: Adult Long-Evans female rats (190–220 g in body weight, Charles River Laboratories) were kept in temperature-controlled animal facilities with a daily cycle of 12 h of light and 12 h of darkness. The experimental and control rats were maintained on commercial laboratory chow and water ad libitum until 18 h before the experiments, when food but not water was removed. All animals were sacrificed between the hours of 09.00 and 11.00. The rats were anaesthetized with pentobarbital sodium (50 mg/kg body weight, i.p.) and tracheostomy was performed with polyethylene tubing to provide a clear airway.

Implantation of Alzet osmotic minipump: The stress of daily injections has the potential to disturb rat feeding activity and adrenocortical hormones, both of which are involved in the regulation of salivary secretory proteins. Therefore, osmotic minipumps were used for delivering the test drug, or, in control animals, the drug vehicle. All surgical equipment was sterilized. Rats were anaesthetized and the ventral skin of the lower abdomen was shaved, cleaned with isopropyl alcohol and swabbed with betadine solution. A small sagittal incision (3 cm in length) was made to one side of the midline, and fascia and muscle carefully separated. An Alzet osmotic minipump (model 2ML2 for 2 weeks or 2ML4 for 4 weeks; Alza Co., Palo Alto, CA, USA) providing 10 or 50 mg/kg/day of amitriptyline (Aldrich Chemical Co. Inc., Milwaukee, WI, USA) was placed inside the abdomen. The muscle was sutured with 5.0 Dermalon (Davis & Geck, Cyanamid Canada Inc., Montreal, Quebec) and the skin was closed with four to five wound clips. Rats were allowed to recover. Assay of blood samples indicated that the delivery of amitriptyline was uniform and steady throughout the experimental period.
was produced by electrical stimulation of the auriculotemporal nerve (AT). The AT, located between the pinna of the ear and the temporomandibular joint, was carefully exposed and bipolar electrodes were placed around it. Stimulation of the parasympathetic innervation to the submandibular gland was accomplished by placing bipolar electrodes around the main excretory duct at a point just below the level at which the lingual nerve (chorda tympani nerve) crosses the duct. The nerves were stimulated electrically by a Grass stimulator (model SD 9) which delivers square-wave shocks (5 ms in duration at a frequency of 16 Hz and an intensity of 4 V).

**Stimulation by isoprenaline:** Isoprenaline (25 mg/kg), a β-adrenergic agonist, was administered intraperitoneally.

**Collection of saliva samples:** For collection of submandibular saliva, a fine polyethylene cannula (Clay-Adams PE 10) was inserted to a distance of 4 mm in the oral opening of one submandibular duct. The saliva was collected from the end of the cannula using disposable micropipettes. Parotid saliva was collected by micropipettes directly from the ipsilateral Stensen's duct severed at the midpoint of its path across the masseter muscle. Both salivary stimulation and collection of samples

![Parotid Saliva](image1)

![Submandibular Saliva](image2)

**FIG. 1.** Change in flow rate of rat saliva evoked by parasympathetic nerve stimulation following chronic administration of amitriptyline (10 mg/kg/day for 2 or 4 weeks). Values are means ± SEM of 4–8 rats.