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Effects of cevimeline on salivation and thirst in conscious rats

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ABSTRACT

Objective: Intraperitoneal injection of a sialogogue, pilocarpine, at high concentrations induces salivation via peripheral pathways and thirst sensation via central pathways. In this study, we report that the effects of another sialagogue, cevimeline, on salivation and water intake in conscious rats differ from those of pilocarpine.

Design: We investigated that effects of peripherally and centrally injected cevimeline on parotid saliva flow rate and water intake in conscious rats. The results were compared with those of pilocarpine.

Results: The intraperitoneal injection of cevimeline induced salivation from the parotid gland, but not water intake. In contrast, the intracerebroventricular injection of cevimeline induced water intake without salivation. The concentration of cevimeline needed to induce salivation by intraperitoneal injection was several 10 times that of pilocarpine, but that needed to induce water intake by intracerebroventricular injection was over a 1000 times greater.

Conclusions: The finding that intraperitoneally injected cevimeline induces salivation without inducing water intake, suggests that the effects on the thirst center in the brain are weaker than those of pilocarpine.

1. Introduction

The numbers of patients complaining about xerostomia has increased recently. Hyposalivation is a side effect of drugs, irradiation for head and neck cancer and Sjögren's syndrome.[1] Pilocarpine and cevimeline, which are muscarinic receptor agonists, are typical sialagogues to treat hyposalivation.[2,3] We reported recently that high concentrations of pilocarpine induced not only salivary secretion, but also water intake and suggested that the former effect was a direct action on the salivary glands, and that the latter effect was elicited through the central nervous system.[4] We think that because of the lack of a blood–brain barrier circumventricular organs may be responsible for the central induction of water intake, because peripherally administrated drugs affect the nuclei directly via the circulation. Muscarinic receptors contain five subunits (M1–M5) and the M3 subtype is related mainly to fluid secretion from salivary glands.[5] In the central nervous system, the M1 and/or the M3 receptor have been reported to be concerned with induction of drinking behaviour.[6,7] Although the selectivities of cevimeline and pilocarpine for the respective muscarinic receptor subtypes are not high,[8] the affinity of cevimeline for the M3 receptor is greater than that of pilocarpine.[9] Therefore, it is possible that cevimeline induces water intake in the same way as does pilocarpine.

Although some previous studies have reported the effects of cevimeline on salivary secretion in anesthetised rats,[8,9] there are no reports about the effects on conscious rats. In the present study we investigated the effects of intraperitoneal and intracerebroventricular injections of cevimeline on

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parotid salivary secretion and water intake in rats, and compared the results with those of our previous studies of pilocarpine.

2. Materials and methods

2.1. Animals

The experiments were conducted on male Wistar rats (350–500 g). All were housed individually in plastic cages under regular light/dark conditions (lights on from 8:00 a.m. to 8:00 p.m.). The temperature was maintained at $23 \pm 1\ ^{\circ}C$ and the humidity was between 60 and 80%. The rats had access to water and laboratory pellets ad libitum, except during the experimental periods. All experimental procedures were approved by the Animal Experiment Committee of Kyushu Dental College.

2.2. Brain surgery

A 24-gauge stainless-steel guide cannula for intracerebroventricular injection of drugs was implanted in each rat under sodium pentobarbital anesthesia (60 mg/kg, intraperitoneally injected), as described in our previous studies.[4,10–12] The cannulas were implanted into the lateral cerebral ventricles 0.8 mm caudal to the bregma, 1.4 mm to the right lateral to midline, and 2.5 mm below the dura matter. The cannulas were fixed to the cranium by means of dental resin and screws. After the surgery, the rats were injected subcutaneously with an antibiotic (Viccillin 10 mg/kg, Meiji, Tokyo, Japan) to prevent infection. Intracerebroventricular injections were performed at least 5 days after the surgery. To be certain that the surgery was successful, we confirmed that rats drank more than 3.5 mL of water during a 10 min period following an intracerebroventricular injection of angiotensin II at 0.03 nmol.

2.3. Drug application

Cevimeline hydrochloride was kindly supplied by Nippon Kayaku Co. Ltd. (Tokyo, Japan). Other drugs were purchased from the following companies: pilocarpine hydrochloride from Kanto Chemical Co. Inc. (Tokyo, Japan), atropine sulfate from Sigma (St. Louis, MO, USA) and angiotensin II from Peptide Institute (Osaka, Japan). The drugs were dissolved in isotonic saline. The concentrations of cevimeline were 12–120 μmol/kg for intraperitoneal injection and 10–3000 nmol for intracerebroventricular injection. Since rats sometimes showed convulsions and in some cases died after intracerebroventricular injection of cevimeline at doses greater than 3000 nmol, higher doses were not used. Atropine was injected intracerebroventricularly 5 min before the cevimeline injections, to block the muscarinic receptors in the brain.

2.4. Measurements of water intake and salivary secretion

Laboratory pellets were removed 1 h before the measurements of salivary secretion and water intakes. The water intakes produced by intraperitoneal and intracerebroventricular injections of cevimeline were measured after 1, 2 and 3 h, to the nearest 0.01 g, by weighing the water bottles.

Cannulation into the parotid gland duct was conducted under sodium pentobarbital anesthesia (60 mg/kg, intraperitoneally injected). A Teflon tube (UT-02, Unique Medical, Tokyo, Japan), connected to a polyethylene tube (SP-10, Natsume, Tokyo, Japan and no. 3, Hibiki, Tokyo, Japan), was inserted into the salivary duct. After recovery from the anesthesia, the end of tube was connected to the second polyethylene tube (no. 3), which was filled with saline. Saliva was collected in 1.5 mL sample tubes, after 1, 2, and 3 h, and saliva volumes were measured to the nearest 1 mg. To make sure the implantation into the duct was successful, we confirmed that at least 100 μL saliva was secreted when rats ate a 100 mg pellet.[11]

2.5. Statistical analysis

The effects of drugs on salivation and water intake were assessed using one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test. For P values less than 0.05 the differences were considered significant, compared with control saline and atropine-coinjected groups.

3. Results

The intraperitoneal injection of cevimeline (40 μmol/kg or higher) induced significant parotid salivary salivation in conscious rats during the first hour (Fig. 1). Higher concentrations of cevimeline (80 and 120 μmol/kg) also induced significant salivation during the second hour after the injection. Further, at 80 μmol/kg, there was a significant salivation during the third hour. It should be noted that while the effects of cevimeline at 120 μmol/kg were less than those at 80 μmol/kg, the animals did not display abnormalities such

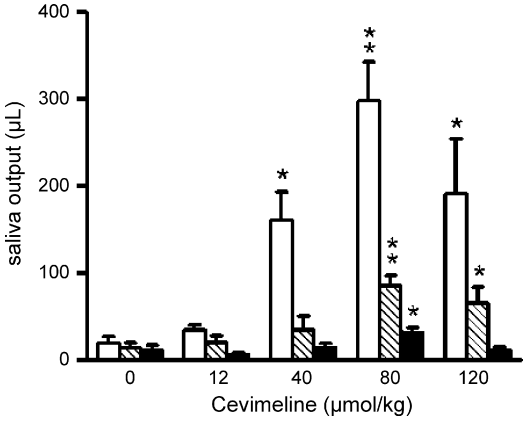

Fig. 1 – Effects of intraperitoneally injected cevimeline on salivary secretion from the parotid gland. The opened, shaded and closed bars represent the volumes of salivary secretion during the first, second and third hours following the injection. Each bar and its error bars represent the means ± S.E.M. The number of rats was 5 in all experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control saline group.