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Long-lasting salivation induced by a novel muscarinic receptor agonist SNI-2011 in rats and dogs

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Abstract

The sialogogic effect of SNI-2011, a novel muscarinic receptor agonist, (±)-*cis*-2-methylspilo [1,3-oxathiolane-5,3′-quinuclidine] hydrochloride, hemihydrate, was compared with that of pilocarpine hydrochloride in a dose range in which the two muscarinic agonists exhibited approximately similar efficacy in eliciting salivation. Pilocarpine (0.66–2.0 mg/kg, i.d.) induced a marked but short-lasting salivation in rats, whereas the salivation induced by SNI-2011 (20–60 mg/kg, i.d.) lasted 1.4- to 1.8-fold longer. In dogs, the sialogogic effect of SNI-2011(1-3 mg/kg, i.v.) also lasted about 2-fold longer than that of pilocarpine (0.1–0.3 mg/kg, i.v.). The plasma SNI-2011 level that caused salivation at a rate of 0.4 ml/min was about 100 ng/ml and higher rates of salivation (over 0.4 ml/min) induced by 1 mg/kg SNI-2011 lasted for about 90 min in dogs. The plasma pilocarpine level that caused salivation at a rate of 0.4 ml/min was about 25 ng/ml and the higher rate of salivation (over 0.4 ml/min) induced by 0.1 mg/kg pilocarpine lasted only for 20 min in dogs. Effective plasma levels of SNI-2011 persisted longer than those of pilocarpine. These results indicate that SNI-2011 may be useful in the treatment of xerostomia because of its long-lasting sialogogic action. © 1997 Elsevier Science B.V.

Keywords: Muscarinic receptor agonists; Salivary gland; Sjögren's syndrome; AF-102B

1. Introduction

Xerostomia is a clinically important symptom experienced by many patients with Sjögren's syndrome or as a side effect of head/neck irradiation. The patients have extremely low rates of saliva flow from salivary glands and so experience difficulties in eating, speaking and swallowing, and in tolerating dentures (Sreebny and Valdini, 1987; Mandel, 1987; Peters, 1989). They frequently suffer from oral and pharyngeal candidiasis and severe tooth decay (Peters, 1989). Several clinical investigations have shown that pilocarpine has promise as a salivary stimulant in normal volunteers (Mandel et al., 1968), patients with xerostomia secondary to radiation therapy (Greenspan and Daniels, 1987; Schuller et al., 1989), and patients with salivary gland dysfunction and/or Sjögren's syndrome (Fox et al., 1986). However, pilocarpine has a short duration of action and at higher doses induces numerous unpleasant side effects, including excessive sweating, nausea, vomiting, diarrhea and arrhythmia (Weaver et al., 1992). These side effects make it difficult to use pilocarpine in the treatment of xerostomia because it must be repeatedly administered at short time intervals to optimize the salivary response and to minimize adverse effects.

A novel muscarinic receptor agonist SNI-2011, (±)-*cis*-2-methylspilo [1,3-oxathiolane-5,3′-qunuclidine] hydrochloride, hemihydrate (Fig. 1), is a rigid analogue of acetylcholine. It has been reported that SNI-2011 elicits saliva secretion from the salivary glands of rats (Iwabuchi and Masuhara, 1994). This compound could be useful in the treatment of xerostomia in patients suffering from the side effects of head/neck irradiation therapy or in association with Sjögren's syndrome. Therefore, the aim of the present study was to characterize the sialogogic effect of

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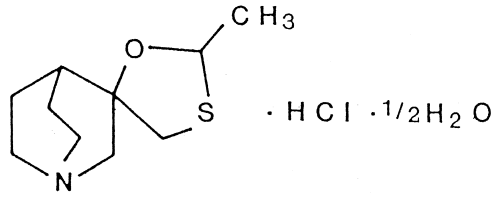

Fig. 1. Chemical structure of SNI-2011.

SNI-2011 in comparison with that of pilocarpine hydrochloride.

2. Materials and methods

2.1. Drugs and reagents

SNI-2011, (±)-*cis*-2-methylspilo [1,3-oxathiolane-5,3′-quinuclidine] hydrochloride, hemihydrate (Snow Brand Milk Products, Tokyo), and pilocarpine hydrochloride (Wako Pure Chemical, Osaka) were dissolved in saline. Deuterated (d_4-)SNI-2011 (Snow Brand Milk Products, Tokyo) was used as an internal standard for SNI-2011 for gas chromatography–mass spectrometry (GC-MS) analysis. Clonidine hydrochloride (Wako Pure Chemical, Osaka) was used as an internal standard for pilocarpine for high performance liquid chromatography (HPLC) analysis. All other reagents were commercial products of analytical grade.

2.2. Animals and general procedure

Wistar rats (40 males; 7 weeks old, 180–200 g) and Beagle dogs (11 males; 8.3–13.0 kg and 13 females; 7.0–11.8 kg) were fasted for 16 h prior to the experiments, but water was available ad libitum. Rats were anesthetized with sodium pentobarbital (80 mg/kg s.c.). Dogs were also anesthetized with sodium pentobarbital (25 mg/kg i.v.) followed by infusion of the anesthetic at a rate of approximately 3 mg/kg/h, which was just sufficient to prevent skeletal muscle movements. The animals were laid on a heating pad maintained at 30°C and the airways were kept open with an endotracheal tube. Rats were laparotomized for the intraduodenal (i.d.) administration of agents. In dogs, a heparinized catheter was inserted into the femoral vein for the injection of agents and for blood sampling.

Saliva secreted by rats was absorbed on dry cotton and weighed over intervals of 10 or 15 min for 180 min after the i.d. administration of SNI-2011 (20, 40 and 60 mg/kg, $n = 5$) or pilocarpine (0.66, 1.3 and 2.0 mg/kg, $n = 5$). In dogs, saliva flowing from the floor of mouth was collected into a tray and weighed over intervals of 10 or 15 min for 90 min after the i.v. injection of SNI-2011 (1, 2 and 3 mg/kg, $n = 3$) or pilocarpine (0.1, 0.2 and 0.3 mg/kg, $n = 3$). Saliva volume was calculated, assuming that 1 ml is equal to 1 g weight of saliva. The half-life of salivation was estimated by fitting the time-elimination curve of saliva volume by a nonlinear least-squares method (Yamaoka et al., 1981).

One group of rats was exclusively prepared for investigating cardiokinetic responses as the side effects induced with SNI-2011 (60 mg/kg, $n = 5$) and pilocarpine (2.0 mg/kg, $n = 5$). Cardiokinetic responses in dogs were monitored during salivation induced by the agonists. Systemic arterial blood pressure was measured with a pressure transducer (TP-400T, Nihon kohden, Tokyo) and an amplifier (AP-621G, Nihon kohden) connected to a catheter inserted into the carotid artery of rats or into the femoral artery of dogs. Heart rate was measured with a heart rate counter (AT-601G, Nihon kohden).

The effective plasma level of SNI-2011 and pilocarpine for induction of salivation was determined in one group of dogs. Blood samples (2.5 ml) were collected at 5, 10, 15, 20, 30, 45, 60, 75 and 90 min after the i.v. injection of SNI-2011 (1 mg/kg, $n = 3$) or pilocarpine (0.1 mg/kg, $n = 3$). At the same time, saliva volume was also measured in these dogs. The heparinized plasma samples were separated from the cells by centrifugation (1900 g, 15 min). Solid NaF (50 mg) was added to the plasma samples used for pilocarpine concentration determination. These samples were stored at −80°C until assayed.

2.3. Measurement of plasma level of SNI-2011 and pilocarpine

The concentration of SNI-2011 free base in plasma was determined by a GC-MS method. A 250 μl aliquot of plasma, 50 μl of d_4-SNI-2011 aqueous solution (500 ng/ml), and 50 μl of 1 N NaOH were placed in a glass centrifuge tube. The mixture was shaken twice with 5.0 ml of *n*-hexane by a mechanical shaker for 10 min, and centrifuged at 700 g for 5 min. The combined organic layer was evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 0.2 ml of methanol, and the dissolved sample was evaporated to dryness as described above. The residue was reconstituted in 20 μl of methanol, and 2 μl of this solution was injected into a GC-MS system. GC-MS was performed on a JMS-505WA mass spectrometer (JEOL, Tokyo) interfaced by a direct inlet system to a HP5890 series II gas chromatograph (Hewlett Packard, CA) with a DB-17 capillary column (15 m × 0.25 mm, J and W Scientific, CA). The injection technique was splitless with an injection port temperature of 280°C. The oven temperature was held at 100°C for 1 min, increased to 200°C at a rate of 40°C/min, held at 200°C for 2.5 min, increased to 280°C at a rate of 50°C/min, and held for 2 min. The mass spectrometer was operated under electron impact at 70 eV in the positive ion mode. With this method, the intra-assay accuracy and precision ranged from 97 to 104% and from 0.6 to 2.8%, respectively, over the range 10.0–500 ng/ml.

The concentration of pilocarpine in plasma was determined by the method of Weaver et al. (1992) with slight modifications. A 0.5 ml aliquot of plasma and 0.5 ml of clonidine solution (30 ng/ml in 50 mM dipotassium hydrogen phosphate) were placed in a glass centrifuge tube. The mixture was extracted with 3.0 ml of dichloromethane by shaking for 10 min, and centrifuged at 700 *g* for 10 min. The organic layer was removed and evaporated to dryness under a stream of nitrogen at 40°C. The residue was reconstituted in 150 μl of 5 mM HCl by vortex-mixing and sonication. The reconstituted sample was vortexed with 2 ml of diethylether, and then centrifuged. After the ether layer was discarded, the ether remaining in the aqueous layer was removed under a stream of nitrogen,

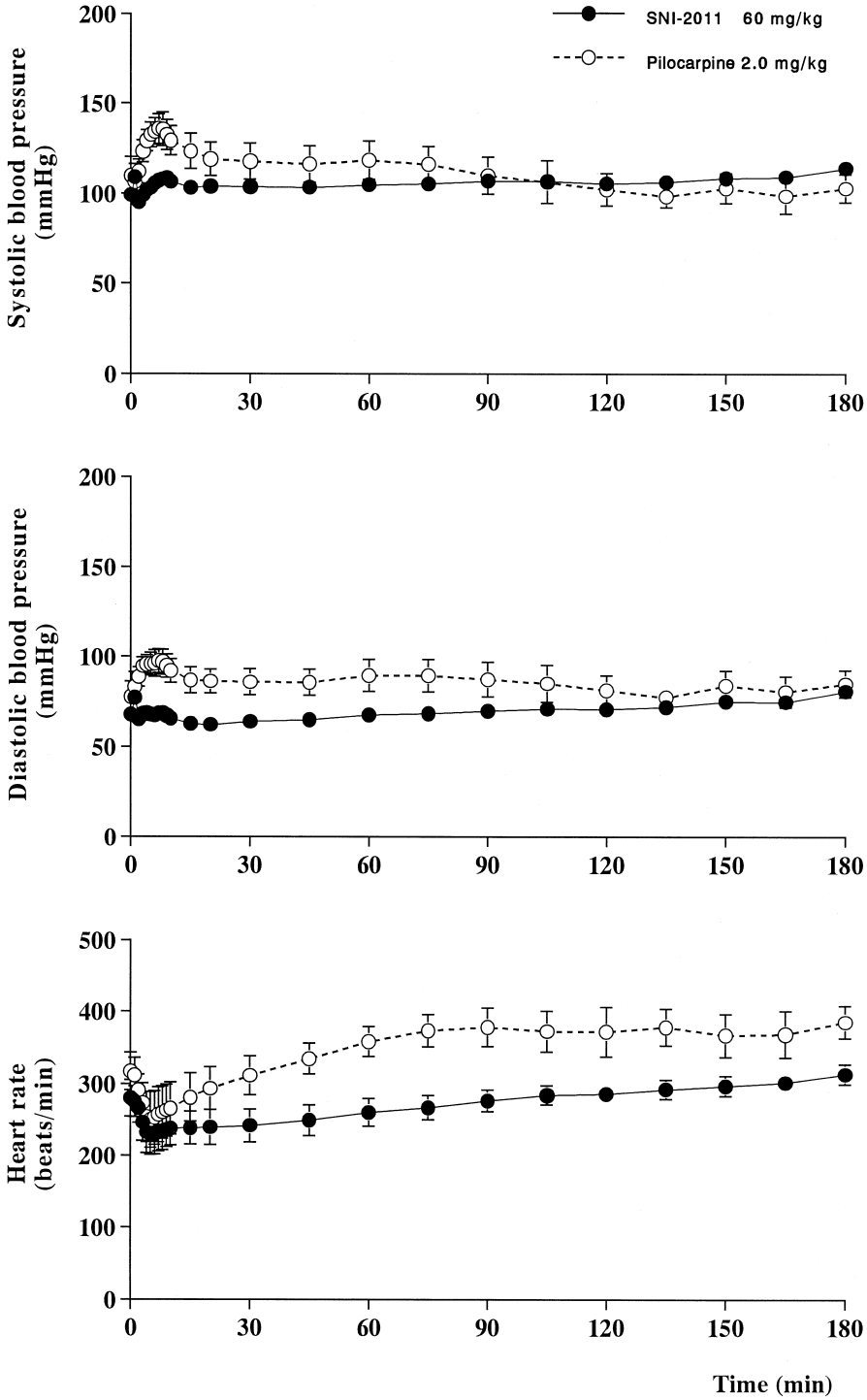

Fig. 2. Effects of SNI-2011 60 mg/kg (●, $n = 5$) and pilocarpine 2.0 mg/kg (○, $n = 5$) on systolic and diastolic arterial blood pressure and heart rate in anaesthetized rats. The drugs were administrated intraduodenally. Each point represents the mean ± S.E.M.

and then 35–100 μl of this aqueous solution was injected into a HPLC system, consisting of a Waters LC module 1 (Nihon Millipore, Tokyo). The samples were separated on a Spherisorb ODS-1 (150 × 4.6 mm i.d., 5 μm particle size; GL Science, Tokyo). The column temperature was maintained at 40°C. The mobile phase consisted of 7 mM potassium dihydrogen phosphate (pH 4.0), acetonitrile, and methanol (55:30:15). The flow rate was 1.1 ml/min, and

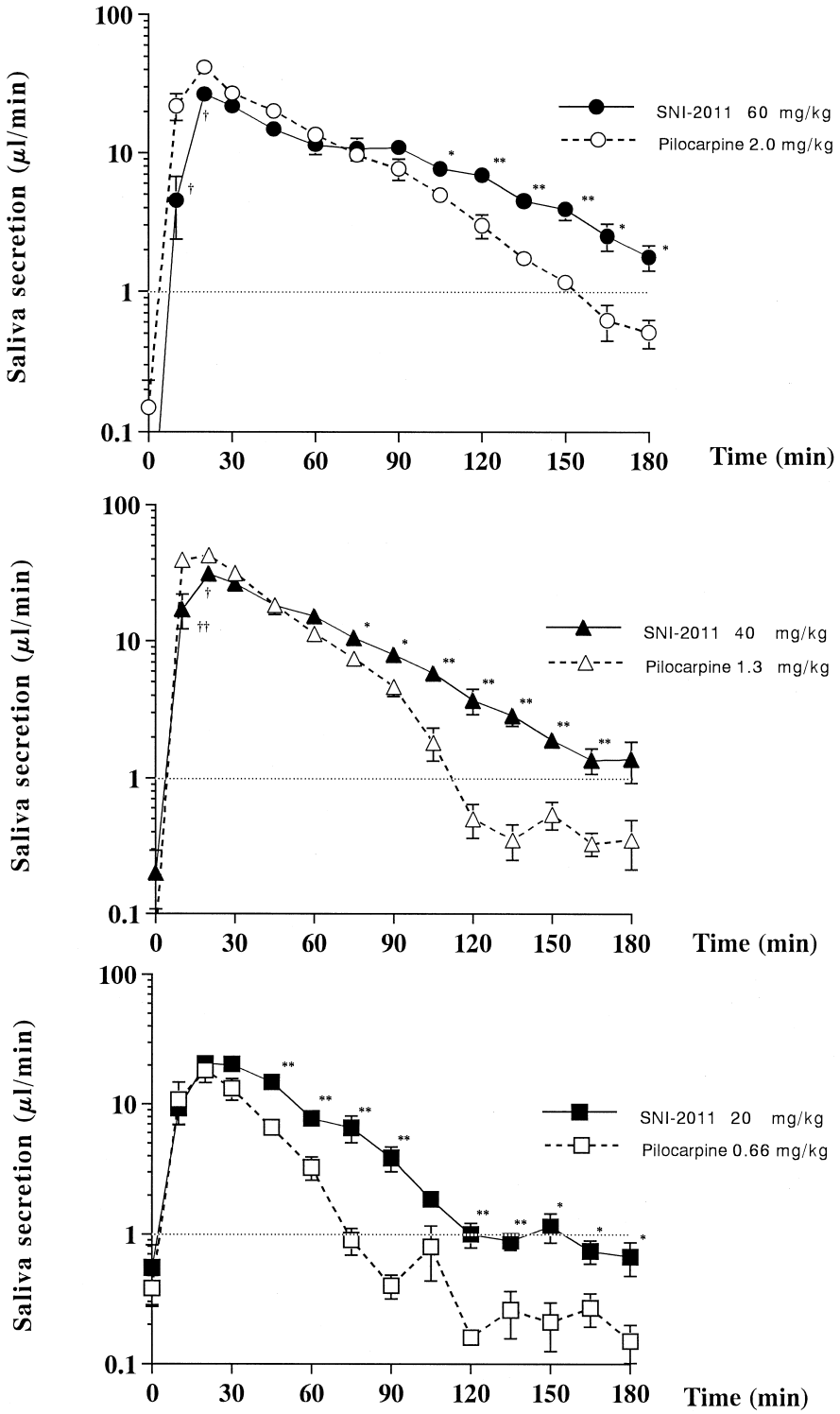

Fig. 3. Comparison of the effects of SNI-2011 (20 (■, $n = 5$), 40 (▲, $n = 5$) and 60 (●, $n = 5$) mg/kg) and pilocarpine (0.66 (□, $n = 5$), 1.3 (△, $n = 5$) and 2.0 (○, $n = 5$) mg/kg) on salivary secretion in anaesthetized rats. The drugs were administrated intraduodenally. Saliva was collected during every 10 or 15 min period. Mean values, calculated as μl/min, are shown with the vertical bars indicating S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly higher, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ significantly lower, when compared with the value in the pilocarpine group.

detection was at 214 nm. With this method, the intra-assay precision was 20.5, 6.3 and 5.1% at the 10.0, 100 and 1000 ng/ml, respectively. The accuracy was 115, 96 and 104% at 10.0, 100 and 1000 ng/ml, respectively.

2.4. Statistical analysis

The results are expressed as the means ± S.E. Statistical comparison was carried out using the unpaired Student's *t*-test for each value. Significance was established at $P < 0.05$ or $P < 0.01$.

3. Results

In rats, hypertension was observed during the first 10 min after the i.d. administration of 2.0 mg/kg pilocarpine, but not after 60 mg/kg SNI-2011 (Fig. 2). Diarrhea was also observed in the rats receiving 2.0 mg/kg pilocarpine. The administration of pilocarpine (0.66–2.0 mg/kg i.d.) induced marked saliva secretion, but salivation lasted only for a short time. In contrast, SNI-2011 (20–60 mg/kg i.d.) resulted in a dose-dependent prolongation of the period of salivation rather than an elevation of the maximum saliva volume (Fig. 3). The saliva volume during the first 20 min after administration of 2.0 mg/kg pilocarpine was larger than that after 60 mg/kg SNI-2011, but the pilocarpine-induced salivation disappeared rapidly. For example, salivation at a rate higher than 1 μl/min lasted 150 and 180 min in rats after the administration of 20 and 40 mg/kg SNI-2011, but it lasted only 75, 105 and 150 min after the administration of 0.66, 1.3 and 2.0 mg/kg pilocarpine, respectively (Fig. 3). After 180 min, the saliva volume elicited by SNI-2011 (20–60 mg/kg i.d.) was more than three-times higher than that elicited by pilocarpine (0.66–2.0 mg/kg i.d.) (Fig. 4). The half-life for salivation in rats given 20, 40 and 60 mg/kg SNI-2011 was 16.4, 26.8 and 39.9 min, respectively. With 0.66, 1.3 and 2.0 mg/kg pilocarpine the half-life for salivation was 11.6, 17.8 and 21.7 min, respectively. Thus, the saliva secretion elicited by SNI-2011 lasted 1.4- to 1.8-fold longer than that elicited by pilocarpine.

In dogs, the temporary hypotension caused by pilocarpine at 0.3 mg/kg i.v. was equivalent to that elicited by SNI-2011 at 3 mg/kg i.v. Reflexive hypertension and tachycardia lasting 45 min were observed after the injection of 0.3 mg/kg pilocarpine, but not after SNI-2011 (Fig. 5). SNI-2011 (1–3 mg/kg i.v.) and pilocarpine (0.1–0.3 mg/kg i.v.) dose dependently induced salivation (Fig. 6). The amount of saliva produced during the first 10 min after the injection of 0.3 mg/kg pilocarpine was greater than that after 3 mg/kg SNI-2011. However, the pilocarpine-induced salivation also rapidly disappeared in dogs. For example, salivation at a rate higher than 0.4 ml/min lasted about 90 min in dogs after 1 mg/kg SNI-2011, but it lasted only 20, 30 and 45 min in dogs after the injection of 0.1, 0.2 and 0.3 mg/kg pilocarpine, respectively (Fig. 6). The half-life for salivation in dogs given 0.1, 0.2 and 0.3 mg/kg pilocarpine was 21.2, 30.9 and 23.5 min, respectively. In contrast, the half-life of salivation in dogs given 1, 2 and 3 mg/kg SNI-2011 was 45.6, 62.2 and 47.2 min, respectively. Thus, the salivation induced in dogs by SNI-2011 lasted about 2-fold longer than that induced by pilocarpine. At 30 min after the injection, the saliva volume in the group given the highest dose of pilocarpine was smaller than that in the group given the lowest dose of SNI-2011 (Fig. 7). Salivation

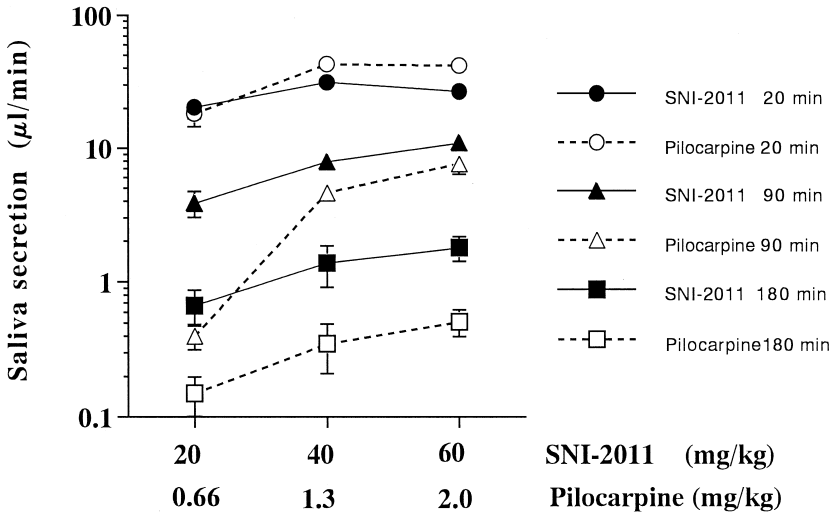

Fig. 4. Salivary secretion at 20 (circle, $n = 5$), 90 (triangle, $n = 5$) and 180 (square, $n = 5$) min after intraduodenal administration of SNI-2011 (solid) and pilocarpine (open) in anaesthetized rats. Mean values, calculated as μl/min, are shown with vertical bars indicating S.E.M.

induced by SNI-2011 at the lowest dose lasted for a long time.

The plasma levels of SNI-2011 and pilocarpine are shown in Fig. 8. The ratios of saliva volume (ml/min)/plasma level (ng/ml) for SNI-2011 (1 mg/kg i.v.) at 10, 30 and 60 min after injection were 1.7/641, 0.8/279 and 0.6/181, while those for pilocarpine (0.1 mg/kg i.v.) were 1.3/34.0, 0.3/20.4 and 0.1/16.2, respectively. Therefore, the effective range of pilocarpine to induce salivation was narrower than that of SNI-2011. For

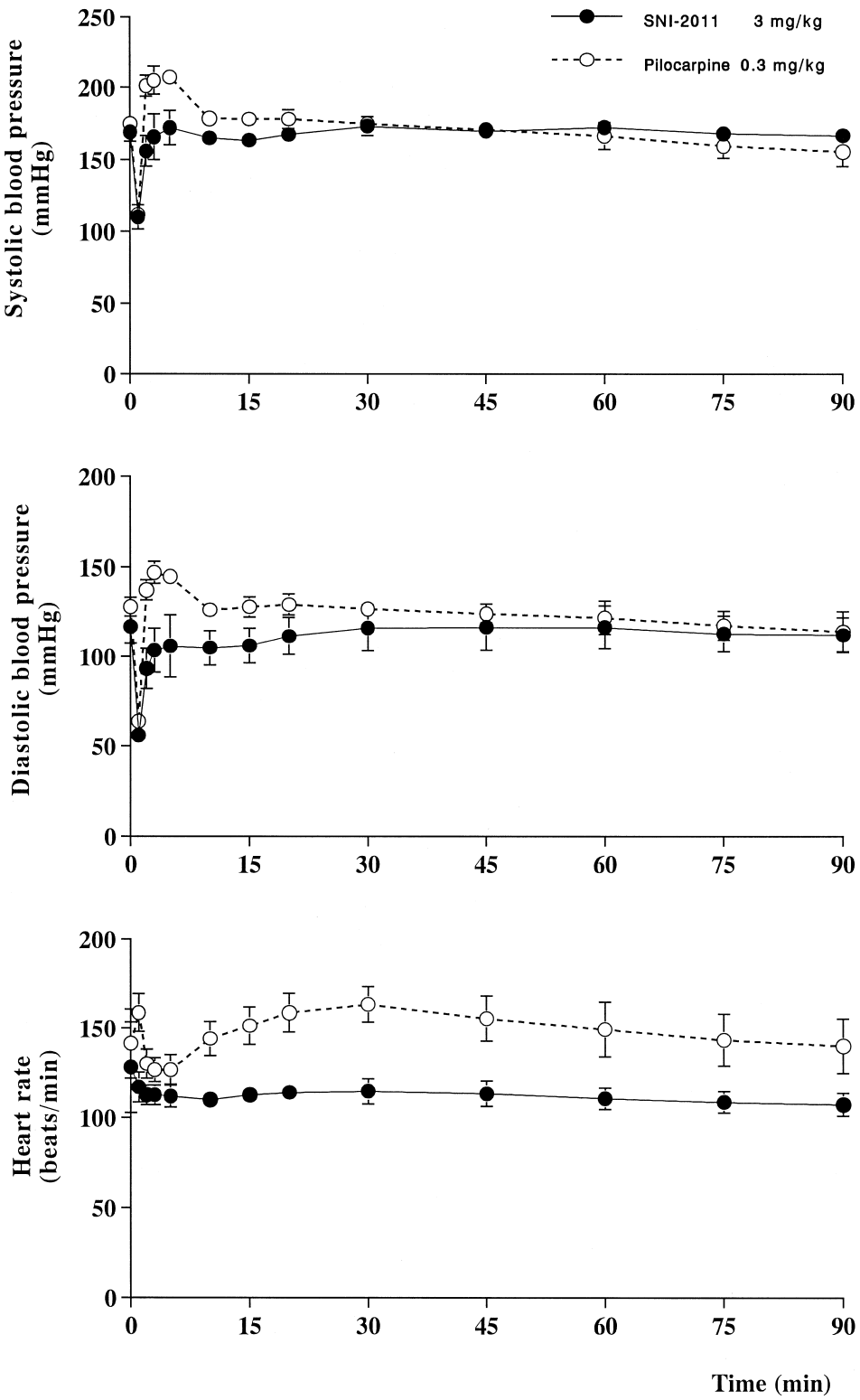

Fig. 5. Effects of SNI-2011 3 mg/kg (●, $n = 3$) and pilocarpine 0.3 mg/kg (○, $n = 3$) on systolic and diastolic arterial blood pressure and heart rates after i.v. injection in anaesthetized dogs. Each point represents the mean ± S.E.M.

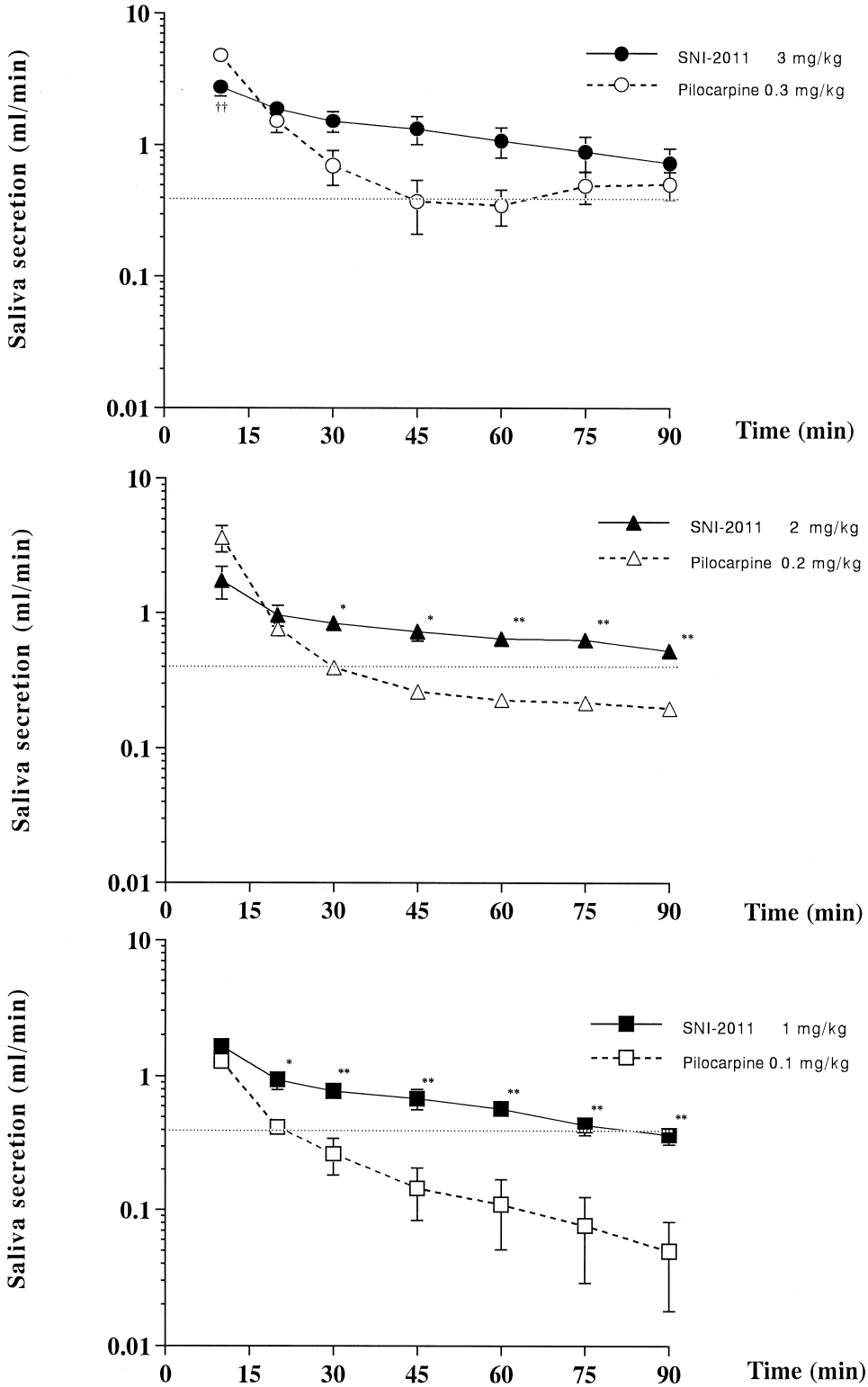

Fig. 6. Comparison of the sialogogic effects of SNI-2011 (1 (■, $n = 6$), 2 (▲, $n = 3$) and 3 (●, $n = 3$) mg/kg) and pilocarpine (0.1 (□, $n = 6$), 0.2 (△, $n = 3$) and 0.3 (○, $n = 3$) mg/kg) on salivary secretion in anaesthetized dogs. The drugs were administered intravenously. Saliva was collected during 10 or 15 min periods. Mean values, calculated as ml/min, are shown with the vertical bars indicating S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly higher, $^{††}P < 0.01$ significantly lower, when compared with the value after pilocarpine administration.

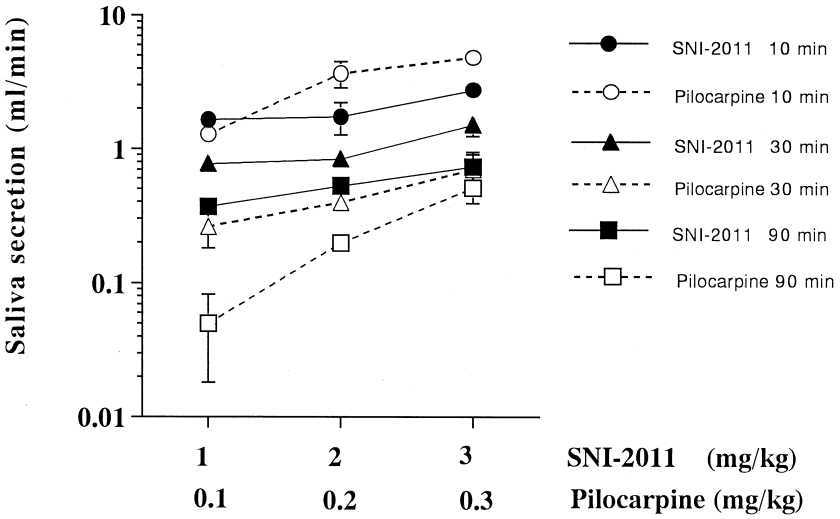

Fig. 7. Salivary secretion at 10 (circle, $n = 3$ or 6), 30 (triangle, $n = 3$ or 6) and 90 (square, $n = 3$ or 6) min after the i.v. injection of SNI-2011 (solid) and pilocarpine (open) in anaesthetized dogs. Mean values, calculated as ml/min, are shown with the vertical bars indicating S.E.M.

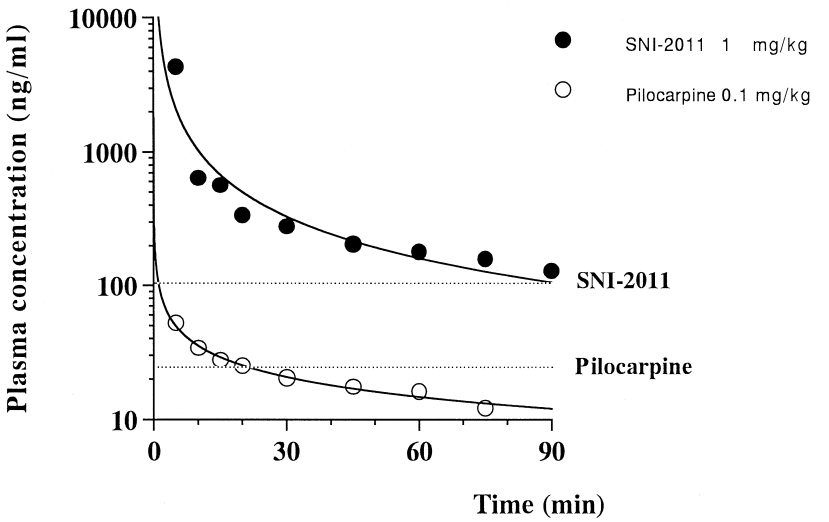

Fig. 8. Changes in plasma concentrations after the i.v. injection of 1 mg/kg SNI-2011 (●, $n = 3$) and 0.1 mg/kg pilocarpine (○, $n = 3$) in anaesthetized dogs. Plasma concentrations of SNI-2011 and pilocarpine causing salivation at a rate of 0.4 ml/min are given by dotted lines. Each point represents a mean value.

instance, the plasma SNI-2011 level that caused salivation at a rate of 0.4 ml/min was about 100 ng/ml and the salivation at a higher rate induced by 1 mg/kg SNI-2011 lasted for about 90 min in dogs. The plasma pilocarpine level that caused salivation at a rate of 0.4 ml/min was about 25 ng/ml and the salivation at a higher rate induced by 0.1 mg/kg pilocarpine lasted only 20 min in dogs.

4. Discussion

Saliva secretion is mainly mediated by the muscarinic receptors in the salivary gland (Laniyonu et al., 1990; Dai et al., 1991; Iwabuchi and Masuhara, 1992). It has been reported that the sialogogic effects of SNI-2011 and pilocarpine are caused by direct stimulation of muscarine receptors in salivary glands (Iwabuchi and Masuhara, 1994). Oral or intravenous administration of pilocarpine has been used frequently to evoke the secretion of saliva in humans (Dawes, 1966; Mandel and Katz, 1971; Greenspan and Daniels, 1987). However, the sialogogic effect of pilocarpine is short-acting and is associated with some adverse effects such as excessive sweating, nausea and diarrhea (Brown et al., 1980; Caulfield and Stubley, 1982; Weaver et al., 1992). Furthermore, in response to pilocarpine, vascular α_1- and cardiac β_1-adrenoreceptors are activated (Wilffert et al., 1983). In rats, the highest dose of pilocarpine used in this study was 2 mg/kg, because this dose induced diarrhea (data not shown) and an unpleasant

hypertension probably mediated by other receptors described above. Therefore, the sialogogic effect of pilocarpine in rats was examined at doses of 0.66, 1.3 and 2.0 mg/kg, given by intraduodenal administration. The sialogogic effect of SNI-2011 was examined at the doses of 20, 40 and 60 mg/kg because, based on the initial salivation, these doses were as effective as 0.66, 1.3 and 2.0 mg/kg pilocarpine. Intraduodenal administration of pilocarpine to rats induced a marked but temporary secretion of saliva. SNI-2011 dose dependently prolonged the duration of salivation, but had little effect on the maximal salivation. The saliva volume at 180 min after the lowest dose of SNI-2011 was higher than that with the highest dose of pilocarpine. The half-life of salivation elicited by SNI-2011 was 1.4- to 1.8-fold longer than that elicited by pilocarpine. Compared with pilocarpine, SNI-2011 produced stable and lasting salivation in rats.

In dogs, the potency of pilocarpine, based on the hypotension immediately after i.v. injection, was about ten times higher than that of SNI-2011. The highest dose of pilocarpine i.v. was selected as 0.3 mg/kg, because the reflexive hypertension and the tachycardia lasted 45 min and also excessive sweating on foot pads was observed (data not shown). Therefore, in order to examine their sialogogic effects, doses of 1, 2 and 3 mg/kg i.v. SNI-2011 and 0.1, 0.2 and 0.3 mg/kg i.v. pilocarpine were chosen. The pilocarpine-induced salivation also disappeared rapidly in dogs. In contrast, the SNI-2011-induced salivation was significantly longer lasting even at the lowest dose used: at 30 min after injection, the saliva volume after the highest dose of pilocarpine was already smaller than that after the lowest dose of SNI-2011. The half-life of salivation elicited by SNI-2011 was approximately one hour in dogs, which was about two-fold longer than that elicited by pilocarpine. The plasma SNI-2011 level for inducing salivation at a rate of 0.4 ml/min was about 100 ng/ml, and the salivation at a higher rate induced by 1 mg/kg SNI-2011 lasted for about 90 min in dogs. The plasma pilocarpine level for inducing salivation at a rate of 0.4 ml/min was about 25 ng/ml and the salivation at a higher rate induced by 0.1 mg/kg pilocarpine lasted only for 20 min in dogs. The range (180–641 ng/ml; 3.6-fold) of plasma levels of SNI-2011 causing salivation was wider than that (20–34 ng/ml; 1.7-fold) of pilocarpine, and effective plasma levels of SNI-2011 persisted for longer than those of pilocarpine. This might be a reason why SNI-2001 had a long-lasting sialogogic activity.

The principal complication of this drug appears to be that of a muscarinic receptor agonist. The muscarinic adverse effects, such as sweating or diarrhea induced by SNI-2011, were less severe than those elicited by 1/10 pilocarpine. It is not likely that the 10-fold higher dose of SNI-2011 necessary to have an effect on salivation similar to that of pilocarpine is a disadvantage because severe undesirable effects have never been observed after SNI-2011 treatment except for mild muscarinic adverse effects. However, it remains to be confirmed by long-term studies whether this new drug is less likely to have unsuitable side effects.

It is concluded that SNI-2011 may be useful in the treatment of xerostomia, because SNI-2011 has a long-acting sialogogic action which may ameliorate the symptoms of xerostomia.

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