Br. J. Pharmacol. (1989), **98**, 101–104

Effects of yohimbine on submaxillary salivation in dogs

Paul Montastruc, Michel Berlan & [1]Jean-Louis Montastruc

Laboratoire de Pharmacologie Médicale et Clinique, Inserm U 317, Faculté de Médecine, 37 allées Jules-Guesde, 31073 Toulouse Cedex, France

1 The effects of yohimbine (0.5 mg kg^{-1} i.v.) on both resting and parasympathetic and sympathetic stimulation-induced submaxillary salivary responses were investigated in the anaesthetized dog.

2 Salivary secretion was increased significantly for a period of 45 min following an injection of yohimbine.

3 Sectioning of the chorda tympani (but not the cervical sympathetic) nerve abolished the yohimbine-induced increase in resting salivary secretion and potentiated that elicited by electrical stimulation of the chorda tympani nerve.

4 These results show that yohimbine increases submaxillary secretion by inhibition of presynaptic α_2-adrenoceptors located on the chorda tympani, which inhibit cholinergic transmission.

Introduction

Several experiments have suggested the involvement of postsynaptic α_2-adrenoceptors in the control of sympathetically and parasympathetically-mediated salivation in the rat (Bylund & Martinez, 1980; 1981; Martinez *et al.*, 1982; Elverdin *et al.*, 1984). In fact, a common side-effect of treatment with α_2-adrenoceptor agonists, especially clonidine, is reduced salivation and a dry mouth. Clonidine reduced submaxillary salivation provoked by either brainstem or peripheral nerve stimulation (Green *et al.*, 1979a); guanabenz, another α_2-adrenoceptor agonist antagonized the secretory responses to noradrenaline, methacholine, substance P (Kaniucki *et al.*, 1984; 1985) and to electrical stimulation of the chorda tympani (Terzic & Stojic, 1986). The decrease in salivation induced by guanabenz was virtually abolished by yohimbine, but not by the α_1-adrenoceptor antagonist prazosin (Terzic & Stojic, 1986). The effects of α_2-adrenoceptor antagonists on salivary secretion, in contrast, have been poorly investigated. Thus, the aim of the present study was to investigate the effects of yohimbine, an α_2-adrenoceptor antagonist (Goldberg & Robertson, 1983) on both resting salivation and that produced in response to parasympathetic and sympathetic nerve stimulation in the anaesthetized dog.

[1] Author for correspondence.

Methods

Animals and general procedure

Dogs of either sex (7 to 15 kg) were anaesthetized with a mixture of chloralose (40 mg kg^{-1} i.v.) plus urethane (500 mg kg^{-1} i.v.). This combination of anaesthetics was chosen from information obtained in preliminary experiments (not shown) which indicated that under either chloralose or, urethane or sodium thiopentone anaesthesia, yohimbine remained ineffective. In view of the potential α_2-adrenoceptor antagonist properties of urethane (Armstrong *et al.*, 1982; Langer & Shepperson, 1982; Moore *et al.*, 1984), this drug was used at low subanaesthetic concentrations, devoid of α_2 activity. The trachea was intubated and the dog artificially respired. Adequate anaesthesia was maintained by injection of 1 mg kg^{-1} chloralose + 5 mg kg^{-1} urethane each hour. Femoral arterial blood pressure and heart rate were measured, respectively, with a Statham P23 Id pressure transducer connected to a Honeywell Bull recorder and a tachocardiometer triggered by an electrocardiogram (lead II) linked to a chart recorder. Body temperature and arterial pH were maintained constant around 38.5°C and 7.42 respectively during each experiment.

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Study of salivary secretion

In the anaesthetized dog, the skin and subcutaneous muscles were incised over approximately 8 cm, along a median path from the angle of the lower mandible to the mandibular arc. The spindle-shaped swelling of the digastric muscle was moved aside. This revealed, on either side of the median raphé, a thin muscle with transverse fibres: the mylo-hyoideus. It was raised on a probe in order to make an incision parallel to the median raphé and 1 cm to the exterior. Just below the mylo-hyoideus, on the deep cervical aponevrosis, near the angle of the mandible, the large lingual nerve was seen crossing the median line and the hypoglossal nerve perpendicularly. Just above the lingual nerve and perpendicular to it under the aponevrosis one can see the grey path of Wharton's duct with, to the inner side, the thinner duct of the sublingual nerve. Following the lingual nerve outwards one can see a thin externally concave nerve come off downwards and to the rear, the chorda tympani nerve, a branch of the facial nerve. This small nerve makes up the third side of a triangle formed in conjunction with the lingual nerve and Wharton's duct. Wharton's duct was catheterized by means of a polythene tube. Under these conditions, three experimental protocols were performed.

Experiment 1: Effect of yohimbine on spontaneous submaxillary salivation Yohimbine (0.5 mg kg^{-1} i.v., 3 ml, $n = 6$) and saline (0.9% NaCl 3 ml, in sham experiments, $n = 6$) were each injected after dissection had been completed, after at least 30 min in resting conditions and salivary secretion from both submaxillary ducts collected every 15 min thereafter for a period of 90 min.

Experiment 2: Effect of yohimbine on parasympathetic salivation A group of experiments (2a, $n = 6$) was performed in order to intestigate the effect of yohimbine on spontaneous salivation with and without the chorda tympani nerve being sectioned. The left chorda tympani nerve was then cut and the effects of yohimbine (0.5 mg kg^{-1} i.v.) on the spontaneous salivation from the intact (right) side compared with the values obtained on the sectioned (left) side.

A second group of experiments (2b) investigated the effect of yohimbine on electrically-induced parasympathetic salivation. After dissection, the left chorda tympani was cut, immersed in a pool of paraffin oil (38°C) and the peripheral end stimulated via bipolar stainless steel electrodes (15 s trains, supramaximal intensity, 0.1 ms, 10 Hz every 15 min, once before and six times after yohimbine (or saline) administration). These parameters of stimulation were chosen since they mimic the salivary secretion found with an intact nerve. Saliva was collected during these 15 s periods of stimulation and during the 15 min period following stimulation. The effects of saline (3 ml, i.v., $n = 6$) and yohimbine (0.5 mg kg^{-1} 3 ml, i.v., $n = 6$) injected at time 0 (i.e. at least 30 min after dissection) were compared on the output of saliva produced by stimulation of the chorda tympani nerve.

Experiment 3: Effect of yohimbine on sympathetic salivation In 6 other dogs, the left cervical sympathetic nerve was cut in the vagal sympathetic trunk and the effects of yohimbine (0.5 mg kg^{-1} i.v.) on the intact (right) side compared with the values obtained on the sectioned and stimulated side according to the protocol described in experiment 2b (15 s trains, supramaximal intensity, 0.1 ms, 10 Hz every 15 min, once before and six times after yohimbine or saline (3 ml) administration).

Drug administration

Yohimbine hydrochloride (Sigma), dissolved in 3 ml saline (0.9%) was injected via the saphenous vein. The dose (0.5 mg kg^{-1}) antagonized the depressor effects of 10 μg kg^{-1} i.v. clonidine on blood pressure, metabolism (plasma free fatty acids and glycerol) and submaxillary salivation (Taouis *et al.*, 1988). Controls received 3 ml saline i.v. as a sham injection.

Statistical analysis

Results are presented as mean values ± s.e.mean. Significance was estimated by use of Student's t test (after one-way analysis of variance). Student's t test for paired comparisons was used when intact and sectioned sides were compared. P values less than 0.05 were considered significant.

Results

Experiment 1: effect of yohimbine on spontaneous submaxillary salivation (Figure 1)

Compared with saline, yohimbine significantly increased salivary secretion during the 45 min following injection. After this time, due to the large individual variations, the increase was not significant. Yohimbine failed to modify blood pressure or heart rate (not shown) eliminating any possibility that variation in salivation arose from changes in blood pressure.

Experiment 2: effect of yohimbine on parasympathetic salivation

Experiment 2a After section of the chorda tympani nerve, the stimulating effect of yohimbine on sponta-

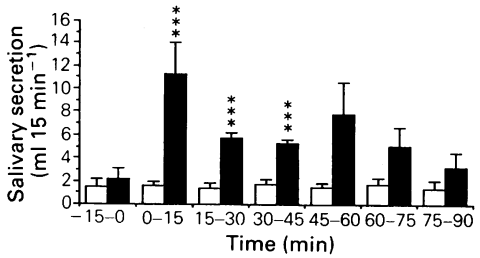

Figure 1 Comparison of the effects of yohimbine (0.5 mg kg^{-1} i.v. at time 0; solid columns) and i.v. saline (open columns) each 3 ml on the salivary secretion (ml 15 min^{-1}) in anaesthetized dogs (Experiment 1). Saliva was collected during the 15 min periods indicated from both submaxillary ducts. ***$P < 0.01$. Mean values are shown with vertical bars indicating s.e.mean; $n = 6$ in each case.

neous salivary secretion was abolished; yohimbine significantly increased salivary secretion from the intact innervated side but not from the sectioned side (Figure 2).

Experiment 2b Unilateral stimulation of the chorda tympani nerve every 15 min for 15 s elicited about 1 ml of saliva irrespective of the time of collection. Yohimbine significantly increased the magnitude of the salivary secretion, to about 2 ml every 15 min following yohimbine injection. After 45 min, the effect of yohimbine was not significant (Figure 3).

Experiment 3: effects of yohimbine on sympathetic salivation

Sectioning of the superior cervical sympathetic nerve failed to modify the spontaneous excito-secretory response to yohimbine (not shown) which was of similar magnitude in the intact (right) and the sectioned (left) sides. Moreover, yohimbine (0.5 mg kg^{-1} i.v.) failed to modify the excitatory response to stimulation of the sympathetic nerve (Figure 4).

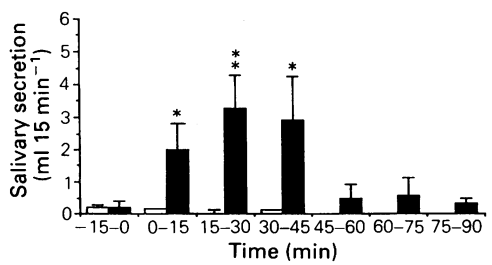

Figure 2 Effect of sectioning the chorda tympani nerve on the enhanced salivary response (ml 15 min^{-1}) produced by yohimbine (0.5 mg kg^{-1} i.v. at time 0) (Experiment 2a). The effects of yohimbine on the intact (right) side (solid columns) were compared with those on the sectioned (left) side (open columns). *$P < 0.05$; **$P < 0.02$. Mean values are shown with vertical bars indicating s.e.mean. $n = 6$.

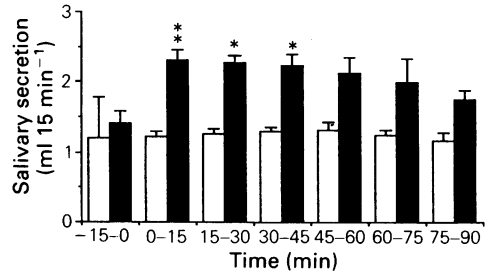

Figure 3 Comparison of the effects of yohimbine (solid columns) and i.v. saline (open columns) on the salivary secretion (ml 15 min^{-1}) elicited by peripheral electrical stimulation of the chorda tympani nerve (15 s trains, supramaximal intensity, 0.1 ms, 10 Hz every 15 min once before and six times after yohimbine (or saline) administration) (Experiment 2b). Stimulation was for 15 s at the beginning of each 15 min period before and after yohimbine (or saline) injection. *$P < 0.05$; **$P < 0.02$. Mean values are shown with vertical bars indicating s.e.mean. $n = 6$.

Discussion

Yohimbine induced a significant increase in the spontaneous salivary secretion in the submaxillary gland of the dog. The effect of yohimbine lasted for 45 min: this duration of action compares with a half-life in man of around 0.6 h (Owen *et al.*, 1987). This stimulating effect of yohimbine, an α_2-adrenoceptor

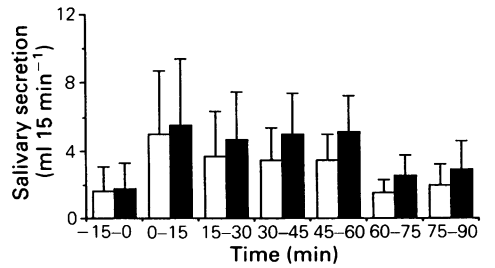

Figure 4 The effect of nerve section on the ability of yohimbine (0.5 mg kg^{-1} i.v. at time 0) to enhance the response of the dog submaxillary gland to stimulation of the superior cervical sympathetic nerve (15 s trains, supramaximal intensity, 0.1 ms, 10 Hz every 15 min once before and six times after yohimbine (or saline) administration) (Experiment 3). The effects of yohimbine on the intact (right) side (solid columns) were compared with those obtained from stimulation of the sectioned (left) side (open columns). Mean values are shown with vertical bars indicating s.e.mean. $n = 6$.

antagonist, in contrast with the inhibitory action of α_2-adrenoceptor agonists (for references see introduction) suggests the involvement of α_2-adrenoceptors in the control of submaxillary secretion.

This effect of yohimbine is dependent on the integrity of the parasympathetic innervation. Thus (1) it is suppressed by sectioning of the cholinergic chorda tympani nerve while (2) yohimbine itself potentiated the increase in salivary secretion in response to chorda tympani stimulation. In contrast, yohimbine did not modify the salivary volume obtained in response to sympathetic nerve stimulation while prior sectioning of the superior cervical sympathetic nerve did not change the magnitude of yohimbine-induced salivation. These results and those of Green *et al.* (1979a) suggest that α_2-adrenoceptors may regulate cholinergic (but not sympathetic) transmission and that yohimbine increases submaxillary secretion by inhibition of presynaptic α_2-adrenoceptors (located on the chorda tympani) which inhibit cholinergic transmission. The role of presynaptic α_2-adrenoceptors in the control of acetylcholine release has been well documented (Langer, 1977). In fact, an effect of yohimbine on postsynaptic inhibitory α_2-adrenoceptors located on the submaxillary gland can be ruled out since yohimbine failed to induce salivary secretion after section of the chorda tympani.

The effect of yohimbine could also have a central component which we cannot evaluate. The central effect is probably of importance since Green *et al.* (1979b) found that clonidine reduces submaxillary salivation evoked by brainstem stimulation in cats. However, the finding that yohimbine remained active during stimulation of the distal end of the chorda tympani (at a frequency which mimicked the salivary secretion found when the nerve was intact) suggests a peripheral action of yohimbine on the α_2-adrenoceptors located on the parasympathetic nerve endings.

The authors acknowledge the technical help of Mr Bernou. Mrs Bontemps prepared the manuscript.

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(Received November 23, 1988
Revised March 9, 1989
Accepted April 21, 1989)