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Serotonin in rat oral tissues: role of 5-HT_1 receptors in sympathetic vascular control

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Abstract

In this study we examined whether the indoleamine, serotonin (5-hydroxytryptamine, 5-HT), is contained in the rat incisor pulp and gingiva as well as its possible role in regulation of blood flow in these tissues. Tissue biochemical analysis, by means of high performance liquid chromatography coupled to electrochemical detection, revealed the presence of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), as well as the catecholamine, dopamine, in both pulp and gingiva. Unilateral surgical sympathectomy or resection of the inferior alveolar nerve failed to affect 5-HT levels in either tissue while dopamine contents in the pulp and gingiva were diminished following sympathectomy. Electrical stimulation of the sympathetic trunk induced a frequency-dependent vasoconstriction in the pulp and gingiva as measured by laser Doppler flowmetry. This vasoconstriction was unaffected by infusion of 5-HT_2 or 5-HT_3 receptor antagonists or dopamine receptor antagonists but it was significantly reduced in both tissues after α_1-adrenoceptor blockade. During this blockade the remaining vasoconstriction induced by high frequency stimulation (16 Hz) was reduced in gingiva by the 5-HT_1 receptor blocker, methiothepin. The results indicate an involvement of 5-HT_1 receptors and α_1-adrenoceptors in the sympathetic vascular control in the gingiva.

Keywords: Afferent nerve; Blood flow; 5-HT (5-hydroxytryptamine, serotonin); 5-HIAA (5-hydroxyindole acetic acid); Dopamine; Gingiva; Dental pulp; Sympathetic nerve; (Rat)

1. Introduction

It is well established that serotonin (5-hydroxytryptamine, 5-HT) causes pain, flare and oedema when applied locally to the blister base on the human forearm (Richardson et al., 1985; Orwin and Fozard, 1986) or when injected intradermally in experimental animals (Arvier et al., 1977). In addition, 5-HT can potentiate the inflammatory effects of substance P (Chahl, 1977). Since substance P-containing nerves are present in many tissues of the body, such as the dental pulp (Olgart et al., 1977) and since the inflammatory effects of 5-HT are considered to be partly mediated via the released neuropeptides from capsaicin-sensitive nerves (Arvier et al., 1977), it is plausible that there is an important interplay in the pulp between the endogenous 5-HT and the sensory nerves. Such a hypothesis is supported by recent findings showing the presence of [^{3}H]5-HT binding sites on perivascularly located nerve fibres in cat and dog pulps (Kim et al., 1992). Thus, the excitation of intradental nerves observed upon local application of 5-HT in cat canine teeth (Olgart, 1974) may be due to the activation of these receptors. Furthermore, 5-HT has been found to sensitize both Aδ- and Aβ-pulpal nerve fibers responding to hydrodynamic stimuli in dog teeth (Ngassapa et al., 1992), possibly via the production of prostaglandins (Hirafuji et al., 1982; Hirafuji and Ogura, 1987). Also, local application of 5-HT on the exposed pulp induces a pronounced vasoconstriction in the pulp (Liu et al., 1990). Taken together these findings make it reasonable to assume that the inflammatory mediator, 5-HT, plays important roles in diseases of the oral tissues such as pulpitis, gingivitis and hypersensitive teeth.

Platelets are considered as the main source of 5-HT in the dental pulp (Olgart and Gazelius, 1978). However, recently Liu et al. (1991) reported the existence of 5-HT-positive neurons in pulps of dog teeth. These neurons may belong to the sensory and/or autonomic

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nervous system since 5-HT has been found in the trigeminal ganglia (Moskowitz et al., 1979) and in the superior cervical sympathetic ganglia (Liuzzi et al., 1977; Verhofstad et al., 1981). The pulp is innervated by both sensory and sympathetic nerves originating from these ganglia (Fried et al., 1988; Kerezoudis et al., 1992, 1993a). However, it is not known whether the 5-HT stored in pulpal neurons can be released upon nerve activation affecting the pulpal blood flow. Therefore the purpose of this study was to examine whether 5-HT is indeed contained in the dental pulp and gingiva of the albino rat and, if so, to investigate its origin and its possible role in the regulation of blood flow in these tissues. For this purpose, both a biochemical and physiological approach were employed. Specifically, the tissue content of 5-HT in the dental pulp and gingiva of rats subjected to unilateral sympathectomy or unilateral inferior alveolar nerve denervation was measured by means of high performance liquid chromatography coupled to electrochemical detection. Under these conditions the tissue content of the catecholamine, dopamine, was also measured in order to examine whether it can be used as a marker for sympathetic nerves. In addition, the effects of 5-HT, dopamine or α_1-adrenoceptor antagonists on changes in blood flow in the dental pulp and gingiva mediated by sympathetic nerve stimulation, were examined by means of laser Doppler flowmetry.

2. Materials and methods

2.1. General preparation

The experiments were performed in male Sprague-Dawley rats (350–450 g) anaesthetized with sodium pentobarbital (Mebumal, Nord Vacc, Skärhamn, Sweden, 50 mg kg^{-1} i.p., further anaesthetic being injected i.v. in doses of 3–6 mg kg^{-1} as necessary). The rats were breathing spontaneously through a cannula placed in the trachea and body temperature was monitored with a rectal thermometer and maintained at 38°C by means of a thermostatically controlled electric blanket. Blood pressure was recorded from the femoral artery and the ipsilateral femoral vein was cannulated for administration of drugs. The animals were placed on their backs with the head fixed to the experimental table and the jaws immobilized by means of a steel rod and dental acrylic cement (Swebond, Svedia, Sweden). The crown surface of the lower incisors was whitened with phosphoric acid (3.77 M) to allow better blood flow recordings and a thin black plastic film was placed between the teeth which then were splinted together by means of a composite dental filling material (Concise, 3M, USA). The experiments were terminated by giving a lethal dose of pentobarbital.

2.2. Chronic sympathetic and afferent nerve denervation

For chronic sympathectomy a surgical approach was employed. The rats ($n = 10$) were pretreated with atropine (Sigma, USA, 0.5 mg kg^{-1} i.p.), anaesthetized with sodium pentobarbital (50 mg kg^{-1} i.p.) and the superior cervical ganglion on one side was removed. The animals were allowed to survive for 10 days after which samples for biochemical analysis were taken (see below).

In another group of rats ($n = 11$) afferent nerve denervation was performed according to the method described by Retief and Dreyer (1969). Briefly, the mandibular bone on one side was exposed by an extraoral lateral approach through an excision in the skin and masseter muscle. The inferior alveolar nerve was exposed by removing a small portion of the bone by means of a small dental round bur under copious irrigation with saline at room temperature and a small segment (2–3 mm) of the nerve was removed. The rats were allowed to survive for 7 days.

2.3. Biochemical analysis

Surgically sympathectomized and afferent nerve denervated rats were killed by a lethal dose of pentobarbital and the head was perfused via the carotid arteries with 100 ml of cold saline (4°C). Incisor pulps and buccal gingiva from the denervated and intact side were immediately removed from the mandible, placed on dry ice, cut in small pieces and weighed. The samples were homogenized by means of an Ultra Turrax (Polytron, Kinematica) or a Sonicator (W-385), in 0.4 M perchloric acid containing 5.26 mM $Na_2S_2O_5$, 1.34 mM ethylenediaminetetraacetic acid (EDTA) and L-α-methyl-3,4-dihydroxyphenylalanine (α-methyl-DOPA) (internal standard), centrifuged at $2910 \times g$ for 20 min at 4°C, and the supernatant was collected and stored at −80°C. Separation of amines was achieved by reverse-phase ion-paired liquid chromatography (150 × 4.6 mm, Nucleosil 5 μm, C-18) with a mobile phase (pH = 3.85 adjusted with glacial acetic acid) consisting of 0.055 M sodium acetate with 0.1 mM 1-octanesulfonic acid, 0.01 mM Na_2EDTA and 11% methanol. The mobile phase was delivered by a high performance liquid chromatography (HPLC) pump (LKB 2150) at 0.8 ml min^{-1} and the chromatograms were recorded on an integrator (SP 4290, Spectra-Physics) and analyzed using commercial software (Winner, Spectra-Physics, San Jose, CA, USA).

2.4. Electrical stimulation of the cervical sympathetic nerves

The cervical sympathetic nerve was unilaterally exposed under a stereomicroscope (Zeiss, Germany),

prepared free from the vagus nerve and cut proximally to the ganglion. The cranial end of the nerve was placed on bipolar silver electrodes, covered with Plastibase (Squibb, UK) to prevent drying and stimulated electrically (4 V, 1 ms, 4 and 16 Hz) for periods of 1 min with a Grass S48 stimulator (Quincy, USA). Before the start of the experimental procedures a resting period of at least 30 min was provided following the end of nerve preparation.

2.5. Recording of blood flow

Changes in blood flow in the ipsilateral mandibular incisor pulp as well as lower gingiva were monitored non-invasively by laser Doppler flowmetry. Fibre-optic probes (custom made, fibre diameter 200 μm with separation 500 μm) were fixed at right angles to and 0.5 mm from the surface of organs (at the distal aspect of the tooth crown), via micromanipulators and connected to Periflux PF2b laser Doppler flowmeters (Perimed, Stockholm, Sweden). Flux zero settings and motility standard calibration of the instruments and fibre-optic probes were made according to the manufacturer. The time constant was set at 1.5 s and bandwidth at 4 kHz in recordings from the tooth pulp and 12 kHz in recordings from the gingiva. To avoid influences of blood pressure on the laser Doppler signal, rats showing a mean arterial blood pressure lower than 80 mm Hg were excluded and all the electrical stimulations were performed under conditions of stable blood pressure and when the laser Doppler recordings showed a stable baseline. Previous studies have indicated a significant correlation in blood flow recordings from oral tissues obtained by laser Doppler flowmetry and other well established methods (Edwall et al., 1987; Kim et al., 1990).

2.6. Experimental protocol and administration of drugs

When stimulation of the cervical sympathetic trunk was employed, at least two control responses were recorded at each stimulation frequency before the administration of drugs. The average value of the corresponding two or more control responses was used for the subsequent statistical analysis. After infusion of each compound (see below), blood flow responses were monitored for a period of at least 80 min during which reproducible vasoconstrictor responses can be obtained (Kerezoudis et al., 1992). The following drugs were used: The competitive α_1-adrenoceptor antagonist, prazosin (Sigma, USA, 100 μg kg^{-1}), was intravenously (i.v.) infused 20 min before electrical stimulation of nerves. In seven animals, prazosin was given as a pretreatment in the beginning of the experiments. The 5-HT receptor antagonist, methysergide (5-HT_1 and 5-HT_2 receptor blocker) (methysergide bimaleate, a gift from Sandoz, Basel, Switzerland, 0.5 mg kg^{-1} i.v.) or ritanserin (5-HT_2 receptor blocker) (Janssen Pharmaceutical, Belgium, 0.5 mg kg^{-1} i.v.), were given 10 min and 20 min respectively before stimulations. The selective 5-HT_3 receptor antagonist, ICS 205-930 (a gift from Sandoz, Basel, Switzerland, 50–100 μg kg^{-1} i.v.) or the potent 5-HT_1 receptor blocker, methiothepin (metitepine maleate, a gift from Hoffmann-La Roche, Switzerland, 300–500 μg kg^{-1} i.v.), was administered at least 15–20 min before nerve stimulation. Methiothepin, which causes some blockage of α_1-adrenoceptors, was also infused in the prazosin-pretreated animals. In some animals the dopamine receptor antagonist, haloperidol (Janssen Pharmaceutical, Belgium, 250 μg kg^{-1}), or the selective dopamine D_1 receptor blocker, SCH-23390 (Schering Co., New Jersey, USA, 0.5 mg kg^{-1}), and dopamine D_2 receptor antagonist, raclopride (Astra, Sweden, 250 μg kg^{-1}), were given i.v. 10 min before nerve stimulation. All drugs were dissolved in 0.9% saline except methiothepin which was diluted in 2% methanol in 0.9% saline. Prazosin was diluted in 5% glucose saline and haloperidol in a minimal amount of acetic acid and subsequently in 5% glucose saline.

2.7. Statistics

All data were stored in an on-line computer and analyzed using commercial software (Perisoft, Perimed). The effects on pulpal blood flow are expressed as percent changes (baseline to peak) and are given as means ± S.E.M. The significance of differences was evaluated with the paired and unpaired Student's t-test. P values less than 0.05 were considered to be significant.

3. Results

3.1. Biochemical analysis

The biochemical analysis revealed the presence of 5-HT, the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) and dopamine in both pulp and gingiva. 5-HT and dopamine tissue contents in the gingiva were higher than those of the pulp (5-HT: 6820 ± 1231 vs. 296 ± 44 pmol g^{-1}, respectively, $P < 0.001$, $n = 21$; dopamine: 104 ± 15 vs. 58 ± 13 pmol g^{-1} respectively, $P < 0.05$, $n = 21$) whereas the 5-HIAA levels were similar in both tissues (gingiva: 430 ± 131 pmol g^{-1}; pulp: 296 ± 44 pmol g^{-1}, $P > 0.05$, $n = 21$). Following sympathectomy, the dopamine contents in the pulp and gingiva were diminished by 79% ($P < 0.05$) and by 65% ($P < 0.01$) respectively (Fig. 1). Chronic surgical sympathectomy failed to affect 5-HT levels in either tissue while it slightly reduced 5-HIAA content in sympathec-

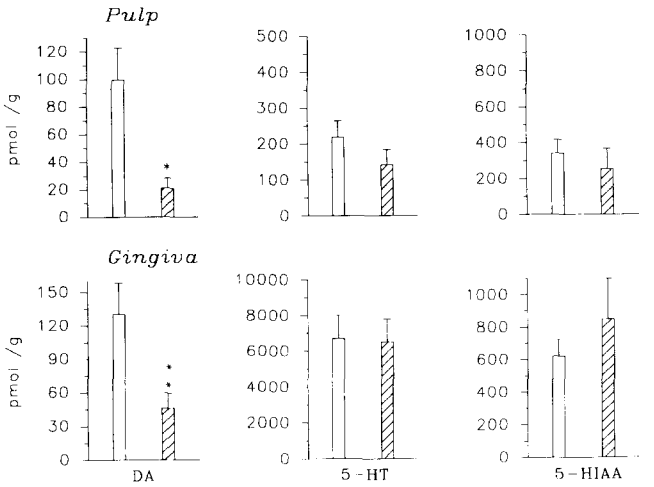

Fig. 1. Effects of unilateral sympathectomy on the content of dopamine (DA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the pulp and gingiva of the rat. Open columns: content in intact contralateral tissues, hatched columns: content in sympathectomized tissues. Bars indicate mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to contralateral control, number of animals $n = 10$.

tomized pulps by 26% ($P > 0.05$, $n = 10$) (Fig. 1). Following afferent nerve lesions, the dopamine content in the denervated pulps was reduced by 50% as compared to intact pulps ($P = 0.05$, $n = 11$) but was unchanged in the gingiva (Fig. 2). Inferior alveolar nerve denervation failed to influence 5-HT and 5-HIAA levels in the pulp and gingiva (Fig. 2).

3.2. Functional experiments

Electrical stimulation of the cervical sympathetic trunk resulted in a vasoconstriction both in the pulp

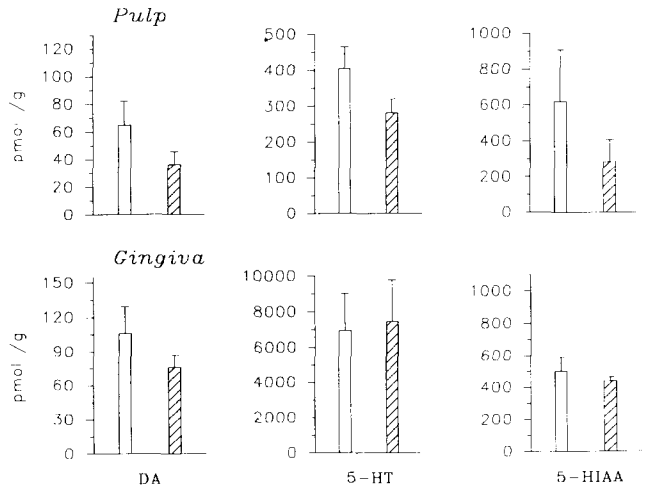

Fig. 2. Effects of unilateral inferior alveolar nerve denervation on the content of dopamine (DA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the pulp and gingiva of the rat. Open columns: content in intact control tissues, hatched columns: content in denervated tissues. Bars indicate mean ± S.E.M., number of animals $n = 11$.

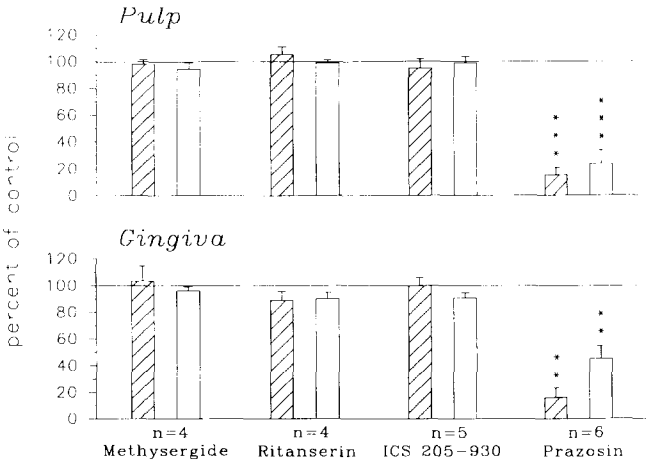

Fig. 3. Effects of 5-HT_2, 5-HT_3 or α_1-adrenoceptor blockers on the vasoconstriction in the pulp and gingiva induced by electrical stimulation of the sympathetic nerve at 4 Hz (hatched columns) and 16 Hz (open columns). Control responses obtained before drug administration are indicated as 100%. All drugs were given intravenously; methysergide 0.5 mg kg^{-1}, 10 min before nerve stimulation; ritanserin 0.5 mg kg^{-1}, 20 min before nerve stimulation; ICS 205-930 100 μg kg^{-1}, 15–20 min before nerve stimulation; prazosin 100 μg kg^{-1}, 20 min before nerve stimulation. Bars indicate mean ± S.E.M.; $^{**}P < 0.01$, $^{***}P < 0.001$, number of animals, $n = 4$–6.

and gingiva; stimulation at 16 Hz produced a greater vasoconstrictor effect in both tissues than those induced at 4 Hz (pulp: 75 ± 4% vs. 57 ± 3% respectively, $P < 0.01$, $n = 15$; gingiva: 81 ± 3% vs. 63 ± 4% respectively, $P < 0.01$, $n = 15$). Infusion of the non-selective 5-HT receptor blocker, methysergide, the 5-HT_2 receptor antagonist, ritanserin, or the 5-HT_3 receptor blocker, ICS 205-930, did not cause any changes in blood pressure or basal blood flow in the pulp or gingiva and they failed to affect the sympathetic vasoconstriction observed at both 4 and 16 Hz in both tissues (Fig. 3) ($n = 4$–5). In contrast, the α_1-adrenoceptor blocker, prazosin, significantly reduced the magnitude of vasoconstriction observed at 4 and 16 Hz in both tissues (pulp: reduction by 85 and 70% respectively, $P < 0.001$, $n = 6$; gingiva: reduction by 81 and 55% respectively, $P < 0.01$, $n = 6$) (Fig. 3). Prazosin caused a slight but insignificant reduction in blood pressure (91 ± 6 mm Hg before and 87 ± 5 mm Hg 20 min after drug treatment). At the same time basal blood flow was slightly lowered in the pulp but was unaffected in the gingiva. When the 5-HT_1 receptor blocker, methiothepin, was administered in untreated animals ($n = 7$), an attenuation of the stimulation-induced vasoconstrictor effects at both 4 and 16 Hz was seen in the pulp and gingiva (Fig. 4). Methiothepin did not cause any changes in blood pressure or basal blood flow in the pulp or gingiva. In the presence of prazosin (100 μg kg^{-1}) the same dose of methiothepin resulted in a reduction by 32% ($P < 0.001$, $n = 7$) of the remain-

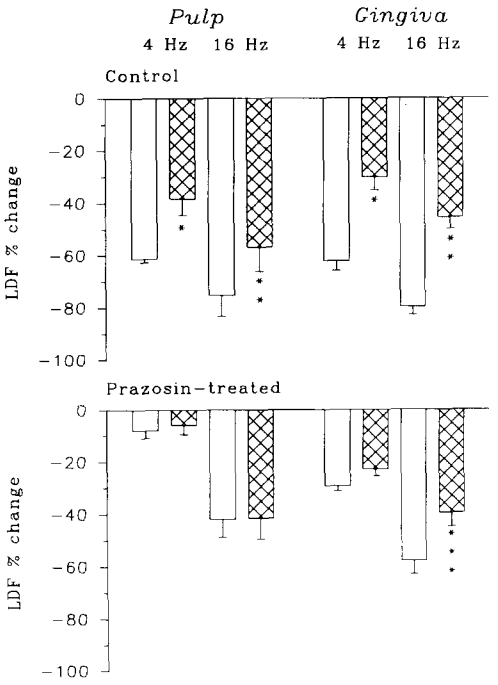

Fig. 4. Effects of the 5-HT_1 receptor blocker, methiothepin 300–500 $\mu g\ kg^{-1}$ i.v.), on the vasoconstriction in the pulp and gingiva induced by electrical stimulation (4 V, 1 ms, 4 and 16 Hz, 1 min) of the cervical sympathetic nerve in the absence ($n = 7$) (upper panel) or in the presence ($n = 7$) of prazosin 100 $\mu g\ kg^{-1}$ i.v. (lower panel). Open columns: responses before, hatched columns: responses after 15–20 min infusion of methiothepin. Bars indicate mean ± S.E.M.; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

ing vasoconstriction induced by the higher frequency (16 Hz) stimulation in the gingiva (Fig. 4). The prazosin-resistant vasoconstriction in the gingiva induced by the 4 Hz nerve stimulation and that induced by both frequencies in the pulp were not affected by methiothepin infusion (Fig. 4). None of the dopamine antagonists had any influence on sympathetic vasoconstriction in the pulp and gingiva. Slow injections ($n = 3$) of the vehicle solutions, isotonic saline, 5% glucose saline and 2% methanol in saline, did not alter the blood pressure or blood flow in the tissues.

4. Discussion

The present study demonstrated, to the best of our knowledge for the first time, the presence of 5-HT and dopamine both in the pulp and gingiva. We also found that the main source of dopamine in these tissues is the sympathetic nerves while we could not obtain any evidence that 5-HT is stored in pulpal nerves. In addition, it seems that 5-HT has a role in the regulation of blood flow in the gingiva but not in the pulp.

Recently, we reported that the incisor pulp and gingiva of the albino rat contain high amounts of noradrenaline which is stored in the sympathetic nerve terminals originating from the superior cervical sympathetic ganglion (Kerezoudis et al., 1992). We now showed that these tissues also contain the precursor of noradrenaline, dopamine. The finding that dopamine levels were significantly reduced in both tissues following removal of the superior cervical sympathetic ganglion suggests that this neurotransmitter is also stored in sympathetic nerves and may be used as a marker for these neurons. The role, however, of dopamine in the sympathetic vascular contol of oral tissues is probably minimal as dopamine receptor antagonists failed to affect sympathetic vasoconstriction in the pulp and gingiva.

It is commonly believed that the sympathetic nerves in the oral region are distributed with branches of the external carotid artery from the external carotid plexus (e.g. Scott and Dixon, 1972). However, functional studies in the cat (Matthews and Robinson, 1980) and histological findings in the rat (Marfurt et al., 1986) prompted the suggestion that the sympathetic nerves originating from the superior cervical sympathetic ganglion, after joining the sensory nerves in the trigeminal ganglion, supply the pulps of the lower teeth exclusively via the inferior alveolar nerve. Our study shed some light on this debate by showing the differential reductions in the dopamine content in the gingiva and pulp after inferior alveolar nerve degeneration. In particular, we found that section of the inferior alveolar nerve failed to modify the dopamine levels in the gingiva and that it resulted in a lower reduction (by 50%) of the dopamine levels in the incisor pulp than that observed following sympathectomy (by 79%). These findings indicate that, in the rat, the sympathetic nerves do not supply the gingiva via the inferior alveolar nerve but rather via another route. For the incisor pulp, our findings indicate that the majority of sympathetic nerves supply the pulp via the afferent nerve and that a small but significant proportion of these nerves arrives via another route, possibly the inferior alveolar vessels.

The finding that the pulp and gingiva contain high amounts of 5-HT and its metabolite, 5-HIAA, is of particular interest. As the 5-HT and 5-HIAA levels in the pulp were moderately, but not significantly, reduced after sympathectomy it may be plausible that sympathetic nerve terminals store some 5-HT in the pulp. In contrast, 5-HT and 5-HIAA in the gingiva seem to be mainly of extraneuronal origin as sympathectomy or section of the afferent nerve supply did not change the levels of these substances. Therefore, the 5-HT-positive neurons observed in the trigeminal ganglia (Moskowitz et al., 1979) and in the superior cervical sympathetic ganglia (Liuzzi et al., 1977; Verhofstad et al., 1981) may supply mainly other tissues such as the cerebral vessels (Griffith and Burnstock, 1983). We have not examined in detail the nature of

extraneuronal pool of 5-HT but platelets are one potential source of 5-HT (Guicheney, 1988; Jernej et al., 1988). Such a possibility is unlikely, however, in our model as the specimens were taken after thorough washing out of the blood from the tissues. In addition, we found in preliminary experiments that the levels of 5-HT contained in the blood volume of the pulp are very low and do not contribute significantly to the total levels of 5-HT in this tissue (mean 5-HT content in pulps without washing: 310, versus after washing: 296 pmol g^{-1}). Another possible source of 5-HT in the pulp and gingiva is the endothelial cells. Considering that these cells are able to take up and accumulate 5-HT (Junod, 1973; Shepro et al., 1975; Burnstock et al., 1988) and subsequently metabolize 5-HT to 5-HIAA (Small et al., 1977), we could not exclude the possibility that 5-HT and its metabolite are stored in this tissue element.

One striking finding of the present study was that the levels of 5-HT in the gingiva were 30-fold higher than those found in the pulp. Pulpal 5-HT levels are in the same range as those reported for other peripheral tissues (Garattini and Valzelli, 1965). This difference suggests the presence of a specific source for 5-HT in the gingiva. In the rat, the gingiva is normally in a state of subclinical inflammation (Kindlova and Scheinin, 1968; Novaes et al., 1991) and contains numerous mast cells (Carranza and Cabrini, 1959). In contrast, the pulp is free from mast cells and normally not inflamed (Radden, 1959; Pohto and Antila, 1970). Since rat mast cells contain high amounts of 5-HT (Benditt et al., 1955), the observed difference in the basal values of 5-HT between the pulp and gingiva may be attributed to the presence of mast cells. It should be mentioned that the high basal values of 5-HT in the gingiva could have masked a possible reduction of the levels of 5-HT in this tissue after sympathectomy or inferior alveolar nerve section. Such a possibility is supported by our functional findings (see below) and by our previous findings showing involvement of 5-HT in the afferent nerve-induced plasma extravasation in the gingiva (Kerezoudis et al., 1993a).

In accordance with our previous work (Kerezoudis et al., 1992, 1993b), we found that electrical stimulation of the cervical sympathetic trunk induced a frequency-dependent, pronounced vasoconstriction both in the pulp and gingiva. The finding that this vasoconstriction was greatly reduced after administration of the α_1-adrenoceptor antagonist, prazosin, but not after infusion of methysergide or ICS 205-930 indicates that it is mediated mainly via postsynaptic α_1-adrenoceptors. In the absence of prazosin, an attenuation of the sympathetically induced vasoconstriction was, however, seen in both tissues after administration of the 5-HT_1 receptor antagonist, methiothepin. This finding may be due to unspecific effects of the drug, since methiothepin exhibits some affinity for α_1-adrenoceptors (Leysen et al., 1981). This notion may also explain the finding that methiothepin failed to affect the remaining vasoconstriction in the pulp as well as that induced by low frequency stimulation in the gingiva after α_1-adrenoceptor blockade. The attenuation of the high frequency-induced, prazosin-resistant vasoconstriction in gingiva observed after administration of methiothepin indicates that 5-HT may play a role in mediating this response. One may argue that this reduction in the magnitude of vasoconstriction is due to an insufficient dose of prazosin and to a partial blockade of the postsynaptic α_1-adrenoceptors. However, such an explanation is unlikely as we have previously shown (Kerezoudis et al., 1993b) that a sufficient α_1-adrenoceptor blockade is achieved in the pulp and gingiva with doses of prazosin (50 μg/kg) even lower than those used in the present study. Furthermore, the finding that administration of methiothepin reduced the prazosin-resistant vasoconstriction observed in the gingiva only at 16 Hz and not that observed at 4 Hz indicates a true pharmacological antagonism and makes the possibility of unspecific effects unlikely. Therefore, it is plausible that 5-HT acting via 5-HT_1 receptors is partially involved in the mediation of the high frequency-induced prazosin-resistant vasoconstriction in gingiva.

The present biochemical findings are seemingly in conflict with our functional results as sympathectomy failed to affect 5-HT levels in the gingiva, whereas a role for 5-HT in the sympathetic nerve-induced vasoconstriction in this tissue was demonstrated. As mentioned previously, one explanation for this discrepancy may be the high basal values of 5-HT in the gingiva which could have masked a possible reduction in the levels of 5-HT in denervated animals. Another possibility is that the sympathetic nerves in gingiva are not involved directly in the synthesis of 5-HT but are taking up and storing the amine from other sources, e.g. via release from platelets or mast cells, due to the chronically inflamed gingiva. Upon activation the sympathetic nerves would then release 5-HT acting on receptors located on the vessels, resulting in vasoconstriction. Nerve uptake and storage may take place because of the similarity in structure between 5-HT and noradrenaline. Uptake of both amines is sensitive to cocaine (cf. Cohen, 1988) and the phenomenon has been shown in other tissues of the body such as the rat vas deferens (Thoa et al., 1969), the dog coronary arteries (Cohen, 1985), the rat tail artery (Szabo et al., 1991) and the guinea-pig basilar artery (Chang and Owman, 1989). Our finding, showing involvement of the 5-HT_1 receptor in the mediation of the prazosin-resistant vasoconstriction in gingiva, seems to be in contrast with the common concept that 5-HT exerts its vasoconstrictor effects via activation of the postsynaptic

5-HT_2 receptors (cf. Vanhoutte, 1982). Tissue and species differences may account for this discrepancy between studies. For example, it has been shown that, in the guinea-pig basilar artery, the amplifying effect of 5-HT on the sympathetic nerve-induced vasoconstriction is mediated by the 5-HT_1 receptor (Chang and Owman, 1989).

In summary, the present study provided evidence for a possible role of 5-HT in sympathetic vasoconstriction in the gingiva. The origin of the available 5-HT still remains unclear, but it is likely to be the blood platelets and/or mast cells. Under pathological conditions, like in inflammation, 5-HT can be released from these cells and may alter the normal function of sympathetic nerves. Whether, under these conditions, 5-HT may exert other actions contributing to the inflammatory process in oral tissues remains to be elucidated.

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