ORAL DISEASES

Oral Diseases (2010) 16, 661–667. doi:10.1111/j.1601-0825.2010.01672.x

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ORIGINAL ARTICLE

Anti-inflammatory action of cholecystokinin and melatonin in the rat parotid gland

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OBJECTIVE: To define the influence of cholecystokinin and melatonin on the inflammatory response of the lipopolysaccharide-exposed rat parotid gland.

MATERIALS AND METHODS: Bacterial lipopolysaccharide was infused retrogradely into the parotid duct. The degree of inflammation three hours postadministration was estimated from the activity of myeloperoxidase, reflecting glandular neutrophil infiltration.

RESULTS: The myeloperoxidase activity of the lipopolysaccharide-exposed gland was 10-fold greater than that of the contralateral gland. Combined with sulphated cholecystokinin-8 (10 or 25 μg kg^{-1}, given twice intraperitoneally) or melatonin (10 or 25 mg $kg^{-1} \times 2$) the lipopolysaccharide-induced response was elevated 4.6- and 3.5-folds at the most. The cholecystokinin-A receptor antagonist lorglumide reduced the inhibitory effect of cholecystokinin-8, while the melatonin 2-preferring receptor antagonist luzindole had no effect on the melatonin-induced inhibition. Unselective nitric oxide-synthase inhibition abolished the increase in myeloperoxidase activity, whereas inhibition of inducible or neuronal nitric oxide-synthase (of non-nervous origin) halved the inflammatory response.

CONCLUSION: Some hormones may contribute to anti-inflammatory action in salivary glands in physiological conditions. They are potential pharmacological tools for treating gland inflammation. The inflammation, as judged from the myeloperoxidase activity, was entirely dependent on nitric oxide-synthase activity, indicating that the hormones directly or indirectly reduced the generation of nitric oxide.

Oral Diseases (2010) **16**, 661–667

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Received 10 October 2009; revised 18 November 2009; accepted 19 November 2009

Keywords: lipopolysaccharide-induced salivary gland inflammation; myeloperoxidase activity; anti-inflammatory; cholecystokinin; melatonin; nitric oxide-synthase inhibitors

Introduction

An inflammatory response develops in the rat parotid gland following the retrograde injection of lipopolysaccharide (LPS) into the parotid duct. The gland is invaded by neutrophils, the myeloperoxidase activity increases, β-defensins are mobilised and oedema develops (Darnell *et al*, 2006).

Salivary glands are not thought to be under the acute influence of gastro-intestinal hormones. Nevertheless, recent experiments reveal that pentagastrin, cholecystokinin and melatonin cause the parotid gland of the rat to secrete proteins and to stimulate protein synthesis by direct action on specific receptors of the gland (Çevik-Aras and Ekström, 2006a,b,c, 2008; Ekström *et al*, 2007). Moreover, the hormonal effects that are exerted are partly dependent on nitric oxide (NO) generated by the activity of neuronal type NO-synthase (Çevik-Aras and Ekström, 2006b,c; Ekström *et al*, 2007).

Salivary gland inflammation may cause hyposalivation and, as a result, the oral health is jeopardised (Grišius and Fox, 1998). Interestingly, both cholecystokinin and melatonin are reported to exert anti-inflammatory action in various experimental conditions (Carrasco *et al*, 1997; Ling *et al*, 2001; Jahovic *et al*, 2003; Reiter, 2003; Barlas *et al*, 2004; Topal *et al*, 2005). Using the rat parotid gland model and LPS-induced myeloperoxidase activity as an index for glandular inflammation, we investigated the anti-inflammatory effect of intraperitoneally administered sulphated cholecystokinin-8 (sCCK-8), one of the molecular forms of cholecystokinin displaying high biological activity (Wu *et al*, 2008), and melatonin. Both sCCK-8 and melatonin reduced the activity of myeloperoxidase and subsequent experiments focused on whether the anti-inflammatory effect was a receptor or a non-receptor

phenomenon. The effect of the two hormones on the myeloperoxidase activity was compared with that of the inhibition of NO synthase and, in this context, attention was paid to the possible involvement of neuronal NO synthase.

Materials and methods

Chemicals

Dapsone (diamino-diphenylsulfone), dimetyl sulfoxide (DMSO) ethylene diamine tetraacetic acid (EDTA), hexadecyltrimethyl ammonium bromide (HETAB), hydrogen peroxide, 3,3′,5,5′-tetramethyl benzidine, lipopolysaccharide (*Escherichia coli* LPS 0111:B4; purified by gel-filtration chromatography), melatonin, sulphated cholecystokinin octapeptide (sCCK-8), itriglumide (CR2945, gastrin/CCK-B receptor antagonist), lorglumide (CR1409, CCK-A receptor antagonist), luzindole (melatonin 2-preferring receptor antagonist), L-NAME (non-selective inhibitor of nitric oxide synthase) and L-NIL hydrochloride (selective inhibitor of inducible nitric oxide synthase, iNOS) came from Sigma Chemicals (St Louis, MO, USA). *N*-PLA (selective inhibitor of neuronal nitric oxide synthase, nNOS) was purchased from Tocris Bioscience, Bristol, UK. Pentobarbitone sodium came from Apoteksbolaget, Stockholm, Sweden. Buprenorphine hydrochloride came from Reckitt Benckiser, London, UK. Ketalar came from Pfizer AB, Sollentuna, Sweden.

Animals

The animal procedures were as humane as possible, performed in accordance with the guidelines laid down by the National Institute of Health in the USA and approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden. A total of 95 Sprague–Dawley rats (Charles River, Sulzfeld, Germany) weighing 296 ± 3 g (mean and s.e.m.) were used; of these, 15 underwent preliminary surgery. The rats were maintained on a pelleted standard diet and tap water *ad libitum*. The preliminary surgery was performed under a combination of pentobarbitone (25 mg kg^{-1}, i.p.) and ketamine (50 mg kg^{-1}, i.m.) and, postoperatively, the animals were given buprenorphine (0.015 mg kg^{-1}, s.c.) as an analgesic. At the terminal experiment, all the animals were anaesthetised with pentobarbitone (55 mg kg^{-1}, i.p.), followed by supplementary doses injected intraperitoneally as required. At the end, the animals, still under anaesthesia, were killed by exsanguination by cutting the abdominal aorta.

Preliminary surgery

The combined parasympathetic and sympathetic denervation of the parotid gland was performed on the right side. The parasympathetic auriculo-temporal nerve was exposed at the point at which it emerges from the skull and avulsed (Khosravani *et al*, 2006). The sympathetic chain was exposed in the neck and the superior cervical ganglion was avulsed (Alm and Ekström, 1977). The wound was sutured. The terminal experiment was performed 2 weeks later.

Preparatory surgery and experimental groups

The anaesthetised animals were placed on a thermostatically controlled blanket with a rectal probe fitted, keeping the body temperature at 38°C. The trachea was cannulated. The parotid duct on the right side was exposed by a small incision externally on the cheek. A syringe filled with LPS (in a concentration of 0.2 mg ml^{-1} sterile saline) attached to a fine polyethylene tube was inserted into the duct and 0.04 ml of the solution was infused slowly towards the gland for a period of 5 s. The tube was then cut open after 5 s to allow overflow, if any, and was closed 2–3 min later by heating, after which the wound was sutured. The gland on the left side was untreated. The duct infusion of LPS on the right side was followed immediately by an intraperitoneal injection (0.3 ml) of either sterile saline, melatonin (10 mg kg^{-1} or 25 mg kg^{-1}), sCCK-8 (10 μg kg^{-1} or 25 μg kg^{-1}), L-NAME (30 mg kg^{-1}), *N*-PLA (30 mg kg^{-1}) or L-NIL (20 mg kg^{-1}). When the effect of the hormone receptor antagonists was tested, an intraperitoneal injection (0.3 ml) of the respective antagonists (lorglumide, 48 mg kg^{-1}, itriglumide, 5.5 mg kg^{-1}, and luzindole, 2 mg kg^{-1}) was given 5 min before LPS. The systemic doses were given once again, after 90 min, but *N*-PLA was only administered once. The dose levels chosen for the hormone receptor antagonists and NO-synthase inhibitors were based on previous studies (Çevik-Aras and Ekström, 2006a,b,c, 2008; Konturek *et al*, 1998). The various study groups and number of animals are shown in Table 1.

About 3 h after the ductal infusion, the parotid glands on both sides were removed, weighed, immediately frozen at −20°C and analysed the following day.

Assay of tissue myeloperoxidase activity

The method used was that of Hillegas *et al* (1990), modified by Kuebler *et al* (1996). Whole gland was homogenised using an Ultra-Turrax at high speed for 30 s in ice-cold potassium phosphate buffer I (50 mM $K_2HPO_4{\cdot}3H_2O$), 13.7 mM HETAB (pH 6.0) 10 times the gland volume. After this, 750 μl of homogenate was centrifuged at 10 000 × *g* for 10 min at 4°C, the supernatant was discarded and the pellet was re-homogenised in an equivalent volume of potassium phosphate buffer II (50 mM $K_2HPO4{\cdot}3H_2O$, 13.7 mM, 10 mM EDTA, pH 6.0) for 5 s. Tubes with 2.9 ml of potassium phosphate buffer III (50 mM $K_2HPO_4{\cdot}3H_2O$, pH 6.0) were put into a shaking bath at 37°C and 30 μl of substrate solution (60 mM 3,3′-5,5′-tetra-methyl benzidine dissolved in DMSO) and 60 μl of 20 mM H_2O_2 were added. The reaction was started by adding a 50 μl sample to the tubes and colour development then took place. The reaction was stopped by adding 60 μl of 0.3 M H_2SO_4. The absorbance of the sample reaction was read at 623 nm and a blank was used to set the reference (Helios Gamma UV-VIS Spectrophotometer, Unicam, Cambridge, UK). The myeloperoxidase assay is not thought to be influenced by other peroxidases (Kuebler *et al*, 1996). Dapsone, a selective inhibitor of peroxidases other than myeloperoxidase, was used to

Table 1 Study groups and wet weights of lipopolysaccharide (LPS)-exposed right parotid glands and left contralateral glands

Groups	n	*Right gland (mg)*	*Left gland (mg)*	*Right gland/Left gland (%)*
Saline	5	215 ± 7.5 ns	211 ± 7	102.1 ± 4.9
LPS	12	201 ± 14***	169 ± 11	119.4 ± 4.4**#
LPS + CCK (10 μg/kg)	5	200 ± 13*	168 ± 13	120.0 ± 5.8*#
LPS + CCK (25 μg/kg)	6	197 ± 14***	180 ± 12	109.8 ± 2.2
LPS + CCK[§] + Lorglumide	8	194 ± 13**	168 ± 11	115.8 ± 3*#
LPS + CCK[§] + Itriglumide	5	201 ± 9*	185 ± 8	108.7 ± 2.8
LPS + MEL (10 mg/kg)	10	181 ± 10*	158 ± 9	114.5 ± 2.2
LPS + MEL (25 mg/kg)	6	207 ± 16*	187 ± 12	110.5 ± 2.6
LPS + MEL[§] + Luzindole	4	180 ± 15**	169 ± 14	107.0 ± 2.4+#
LPS + L-NAME	8	190 ± 14**	180 ± 13	105.6 ± 2.3 + +#
LPS + *N*-PLA	5	178 ± 6**	166 ± 5	107.6 ± 4.1 + #
LPS + L-NIL	6	154 ± 5***	139 ± 6	111.2 ± 2.6
Right glands chronically denervated				
Saline	5	93 ± 6***	180 ± 19	52.4 ± 2.4
LPS	5	119 ± 11***	189 ± 16	63.3 ± 4.1
LPS + *N*-PLA	5	126 ± 12***	192 ± 18	67.0 ± 6.6

Paired comparisons between LPS-exposed glands and contralateral glands were made by using Student's *t*-test, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$; ns is non-significant. #Group comparisons were made by using ANOVA, *$P < 0.05$, **$P < 0.01$ compared with saline-infused group; +$P < 0.05$, + +$P < 0.001$ compared with LPS-infused group. Combined parasympathetic and sympathetic denervation of the right gland was performed two weeks in advance. [§] Denotes that the high dose was chosen. Lorglumide is a CCK-A receptor antagonist, itriglumide a CCK-B receptor antagonist and luzindole a melatonin 2-preferring receptor antagonist. L-NAME is a non-specific NO-synthase inhibitor, while *N*-PLA is a neuronal type NO-synthase inhibitor and L-NIL is an inducible NO-synthase inhibitor. Values are means and s.e.m., and *n* is the number of rats (and glands).

ascertain the specificity of the assay (Thomas *et al*, 1994). One unit of enzyme activity was defined as the amount of myeloperoxidase present that caused a change in absorbance of 1.0 min^{-1} at 37°C. Myeloperoxidase activity was expressed as total activity per gland (units per whole gland per minute).

Statistics

Paired comparisons between the right and left parotid gland of the same animal were made using Student's *t*-test. For comparisons between groups, the right gland was expressed as a percentage of the left gland and, based on percentage figures and using one-way analysis of variance (ANOVA), followed by Fisher's protected least-significant difference, statistical significances were calculated. Values are means and s.e.m. $P < 0.05$ was considered significant.

Results

Gland weights

The duct infusion of saline alone did not influence the wet weights of the glands. In contrast, all the LPS-infused glands in the various study groups were significantly heavier, by 6–20% ($P < 0.05$–$P < 0.001$), than the non-infused glands on the contralateral side (Table 1).

LPS-induced myeloperoxidase activity: effects of sCCK-8 and melatonin with and without receptor antagonists

There were no statistical differences in the total activity of myeloperoxidase between the left contralateral glands of the various groups. The mean total activity in all the examined contralateral glands was 3.1 ± 0.2 ($n = 80$) units per gland per minute.

About 3 h after the duct infusion of LPS, the myeloperoxidase activity of the right, LPS-exposed gland was 10 times higher than that in the left contralateral gland ($n = 12$, $P < 0.001$). An infusion of saline alone had no effect; the myeloperoxidase activity of the right gland was 1.1 times that of the contralateral gland ($n = 5$), (Figure 1).

Lipopolysaccharide combined with sCCK-8 10 μg $kg^{-1} \times 2$ ($n = 5$) and 25 μg $kg^{-1} \times 2$ ($n = 6$) resulted in significantly smaller increases than LPS alone ($P < 0.01$), 4.6 times and 3.5 times respectively. When the CCK-A receptor antagonist lorglumide was given in addition, the effect of sCCK-8 (25 μg $kg^{-1} \times 2$) was diminished ($n = 8$, $P < 0.05$), while the increase in myeloperoxidase activity was 6.3-fold. When the CCK-B receptor antagonist itriglumide was given instead, the effect of sCCK-8 (25 μg $kg^{-1} \times 2$) was in the same range as the effect in the absence of the CCK-B receptor antagonist ($n = 5$), the increase in myeloperoxidase activity was now 4.3-fold (Figure 1).

Lipopolysaccharide combined with melatonin 10 mg $kg^{-1} \times 2$ ($n = 11$) and 25 mg $kg^{-1} \times 2$ ($n = 6$) also resulted in significantly smaller increases than to LPS alone ($P < 0.05$ and $P < 0.01$ respectively), 7.5-fold and 3.5-fold. The reduction in myeloperoxidase activity in the presence of melatonin, 25 mg $kg^{-1} \times 2$, persisted when the melatonin receptor antagonist luzindole was given in addition ($n = 4$); the increase in activity was 2.3-fold (Figure 2).

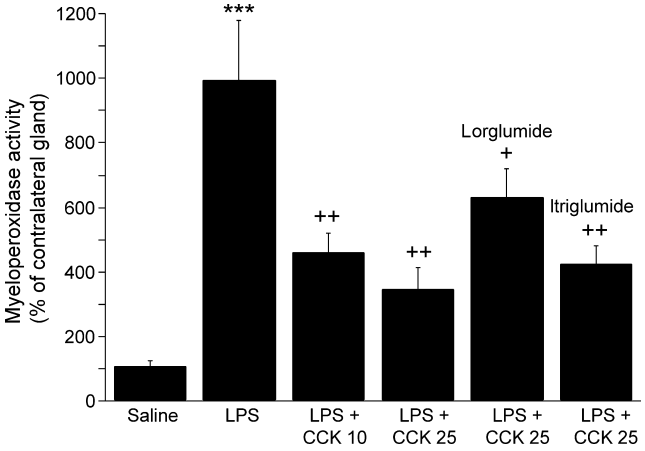

Figure 1 The effects of saline- or lipopolysaccharide (LPS)- duct infusion alone or in the presence of sulphated cholecystokinin-8 (sCCK-8) (10 μg kg^{-1}, i.p., × 2, $n = 5$ and 25 μg kg^{-1}, i.p., × 2, $n = 6$) with and without either lorglumide (48 mg kg^{-1}, i.p., × 2, $n = 8$) or itriglumide (5.5 mg kg^{-1}, i.p., × 2, $n = 5$). The columns represent means of the myeloperoxidase activity of the treated gland expressed in percent of that of the untreated (contralateral) gland (vertical bars are + s.e.m.). *$P < 0.05$, ***$P < 0.001$ compared with the saline-infused group. +$P < 0.05$, + +$P < 0.01$ compared with the LPS-infused group

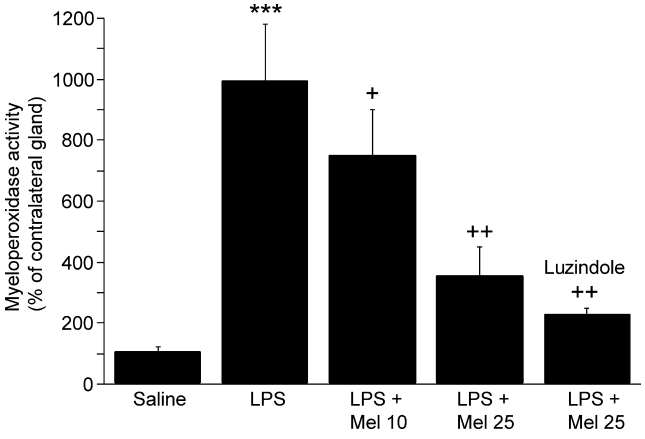

Figure 2 The effects of saline- or lipopolysaccharide (LPS)- duct infusion alone or in the presence of melatonin (10 mg kg^{-1}, i.p., × 2, $n = 10$ and 25 mg kg^{-1}, i.p., × 2, $n = 6$) with and without luzindole (2 mg kg^{-1}, i.p., × 2, $n = 4$). The columns represent means of the myeloperoxidase activity of the treated gland expressed in percent of that of the untreated (contralateral) gland (vertical bars are + s.e.m.). ***$P < 0.001$ compared with the saline-infused group. +$P < 0.05$, + +$P < 0.01$ compared with the LPS-infused group

LPS-induced myeloperoxidase activity: effects of non-specific and specific NO-synthase inhibitors

The LPS-induced increase in myeloperoxidase activity was markedly affected by the non-specific NO-synthase inhibitor L-NAME. The activity was 2.4 times higher than that of the contralateral glands ($n = 8$) and was, in fact, not significantly different from that of the glands exposed to a duct infusion of saline. The myeloperoxidase activity showed higher figures in the presence of the inducible NO-synthase inhibitor L-NIL ($n = 6$) and the neuronal type NO-synthase inhibitor *N*-PLA ($n = 5$) respectively and was 4.9 times and 4.4 times higher than that of the contralateral glands, but the activity was still significantly lower than that in response to LPS alone ($P < 0.05$) (Figure 3).

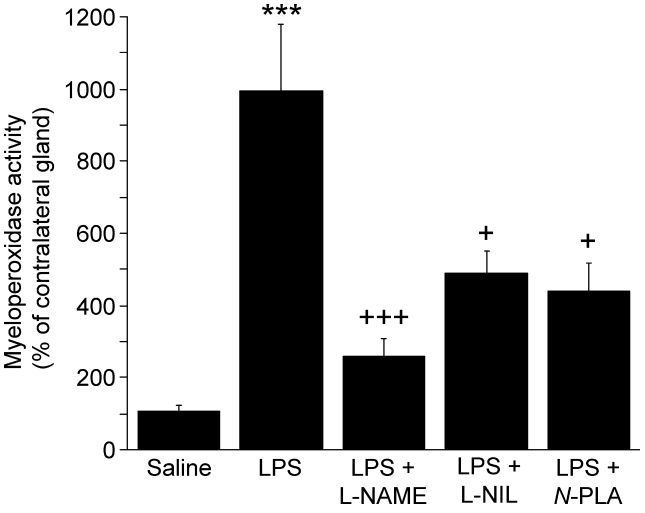

Figure 3 The effects of saline- or lipopolysaccharide (LPS)- duct infusion in the presence or the absence of NO-synthase inhibitors [the unspecific NO-synthase inhibitor L-NAME, 30 mg kg^{-1}, i.p., × 2 ($n = 8$), the inducible NO-synthase inhibitor L-NIL, 20 mg kg^{-1}, i.p., × 2 ($n = 6$) or the neuronal NO-synthase inhibitor *N*-PLA, 30 mg kg^{-1}, i.p. ($n = 5$)]. The columns represent means of the myeloperoxidase activity of the treated gland expressed in percent of that of the untreated (contralateral) gland (vertical bars are + s.e.m.). ***$P < 0.001$ compared with the saline-infused group. +$P < 0.05$, + + +$P < 0.001$ compared with the LPS-infused group

Observations on denervated glands

As might be expected, the duct-infused parotid glands subjected to chronic parasympathetic and sympathetic denervation were significantly ($P < 0.01$) lighter than those on the non-denervated side in terms of wet weight (Table 1); in the group of saline infusion lighter by 48%, in the group of LPS infusion by 37% and in the group of LPS infusion combined with an intraperitoneal injection of the neuronal type NO-synthase inhibitor *N*-PLA by 33%. At the same time, the figures for weight difference, expressed as percentages, were lower in the groups exposed to LPS (with or without *N*-PLA) than in the group exposed to saline alone, but the difference was not statistically significant.

The total myeloperoxidase activity of the glands on the non-operated side was 2.4 ± 0.3 ($n = 15$) units per gland per minute; there was no difference between the glands on the non-operated side in the rats in the three groups that were studied. The myeloperoxidase activity in the atrophied glands exposed to the infusion of saline was the same as in the contralateral glands, but it was 16 times higher in response to the infusion of LPS ($P < 0.001$, $n = 5$); the larger increase in denervated glands compared with non-denervated glands (see above) was probably due to the fact that the atrophied glands were exposed to a larger amount of LPS per gland unit. Moreover, the increase in myeloperoxidase

activity was lower in the presence of *N*-PLA ($n = 5$, $P < 0.05$), where it was 7.2 times higher (Figure 4).

Discussion

Following LPS administration, systemically, the *in vivo* salivary secretion from the rat submandibular gland is hampered (Lomniczi *et al*, 2001). Likewise, administration of LPS *in vitro* reduces the mucin synthesis of the acinar cells of the rat sublingual gland (Slomiany and Slomiany, 2002). Myeloperoxidase is a natural constituent of primary granules of neutrophils. There is a direct relationship between tissue myeloperoxidase activity and the number of neutrophils (Bradley *et al*, 1982). The expected increase in myeloperoxidase activity in the parotid gland following the infusion of LPS was markedly reduced, in a dose-dependent manner, by melatonin and sCCK-8 administered intraperitoneally. Immunoblotting demonstrates the presence of the melatonin 1 and 2 types of receptor, as well as the presence of the CCK A and B types of receptor, in the rat parotid gland (Çevik-Aras and Ekström, 2006a, 2008). The inhibitory effect of melatonin persisted in the presence of the antagonist luzindole. The effect of melatonin is therefore not exerted via melatonin 2 receptors. Although luzindole is characterised as a melatonin 2-preferring antagonist, it also has some affinity to melatonin 1 receptors (Dubocovich *et al*, 1998). It may therefore be premature to decide whether the inhibitory action exerted by melatonin in the present study was a receptor- or a non-receptor-mediated phenomenon. In contrast, the inhibitory effect of sCCK-8 involved CCK-A receptors (the inhibitory effect of sCCK-8 was diminished by lorglumide) but not CCK-B receptors (the inhibitory effect of sCCK-8 was not diminished by itriglumide). Alongside the diminished myeloperoxidase response to LPS in the presence of melatonin and sCCK-8, the expected gain in gland wet weight was reduced, a further sign of the anti-inflammatory action of melatonin and sCCK-8.

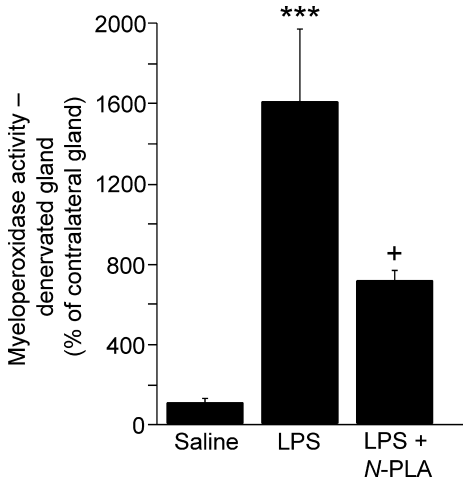

Figure 4 Glands subjected to chronic combined parasympathetic and sympathetic denervation. The effects of saline- or lipopolysaccharide (LPS)-duct infusion in the presence or the absence of the neuronal NO-synthase inhibitor *N*-PLA, (30 mg kg^{-1}, $n = 5$). The columns represent means of the myeloperoxidase activity of the treated gland expressed in percent of that of the untreated (contralateral) gland (vertical bars are + s.e.m.). ***$P < 0.001$ compared with the saline-infused group. +$P < 0.05$ compared with the LPS-infused group

An anti-inflammatory action by exogenous melatonin via reduced neutrophil infiltration and the expression of inducible NO have been demonstrated in the rat liver (Sewerynek *et al*, 1996; Wu *et al*, 2001), together with the inhibition of inducible NO synthase activity in the paw and pleura of the rat (Cuzzocrea *et al*, 1997). It has been suggested that melatonin acts intracellularly as a free radical scavenger and antioxidant to neutralise reactive oxygen and nitrogen compounds (Reiter *et al*, 2000a). Both sulphated and desulphated forms of CCK-8 exert inhibitory effects *in vitro* on the chemotaxis and mobility capacity of human neutrophils; these phenomena are mediated via CCK-A receptors (Carrasco *et al*, 1997). Moreover, sCCK-8 diminishes the LPS-induced increase in inflammatory cytokines involved in NO generation, *in vivo* in the heart and lung of the rat (Ling *et al*, 2001).

The increase in the myeloperoxidase activity of the parotid gland upon LPS exposure was dependent on NO generation, as it was entirely abolished by the non-selective NO-synthase inhibitor L-NAME. Although LPS-induced inflammation is usually associated with inducible NO synthase (Reiter *et al*, 2000b), the present study also revealed the involvement of the neuronal type of NO synthase. Evidently the neuronal type of NO synthase was of non-nervous origin, as it was still mobilised in chronically denervated glands. Whether melatonin and sCCK-8 achieved their inhibitory effect on glandular NO production in the LPS-exposed parotid gland by a direct or indirect action on inducible NO synthase and neuronal type NO synthase is currently unknown. However, melatonin has been shown to inhibit the LPS-induced activity of inducible NO synthase in the liver and lungs of rats (Crespo *et al*, 1999).

Interestingly, the neuronal type of NO synthase of non-nervous origin in the rat parotid gland has previously been shown to be involved in secretion and protein synthesis in response to β-adrenoceptor agonists and the hormones currently being studied (Ekström *et al*, 2007; Çevik-Aras and Ekström, 2008). Bacterial products (LPS) and/or cytokines are thought to be needed to trigger the inflammatory response (Morris and Billiar, 1994). The activation of neuronal NO synthase in response to β-adrenoceptor agonists, cholecystokinin or melatonin is therefore not likely to initiate any inflammation by itself.

The dose levels of melatonin and sCCK-8 used in the current study were in the same range as those in some other studies revealing anti-inflammatory action by the hormones (Cuzzocrea *et al*, 1997; Crespo *et al*, 1999; Ling *et al*, 2001) and they must be characterised as supraphysiological. However, melatonin, cholecystokinin and also gastrin (using the same CCK receptors as cholecystokinin), at low concentrations, could hypothetically act in concert and, together with other

released anti-inflammatory substances, attenuate the inflammatory tissue response (Reiter *et al*, 2000a). With respect to salivary glands, the anti-inflammatory action of hormones may not only be restricted to the gland itself. Both melatonin and gastrin have been shown to appear in the saliva and the amounts increase during a meal (Cutando *et al*, 2007; Czesnikiewics-Guzik *et al*, 2007). When secreted, they may thus be a part of the oral defence. Saliva contains particularly high concentrations of melatonin, about 25% of that in the plasma (Laakso *et al*, 1990). Melatonin has been suggested for the treatment of inflammation in various clinical conditions, including cancer (Giannoulia-Karantana *et al*, 2006), rheumatoid arthritis (Korkmaz *et al,* 2008), urinary bladder cystitis (Topal *et al*, 2005) and postoperative oral surgery (Cutando *et al*, 2007); for local administration in the latter case. Moreover, melatonin can be used to reduce inflammation produced as a side-effect in drug therapy (Reiter *et al*, 2002). Salivary gland inflammation can therefore be added to the list of therapeutic options for melatonin (and cholecystokinin/gastrin) in oral diseases (see Gòmez-Moreno *et al*, 2009).

Acknowledgements

This study was supported by the Swedish Science Council (05927) and the LUA/ALF agreement (ALFGBG-11907).

We wish to thank Mrs Ann-Christine Reinhold for her excellent assistance. We declare no conflict of interest.

Author contributions

This study was prepared as part of Dr H Çevik-Aras' PhD thesis mentored by Professor J Ekström.

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