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Calcitonin-gene related peptide is a potent inducer of oedema in rat orofacial tissue

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ABSTRACT

Background and aims: This study aimed to assess the potential of calcitonin-gene related peptide (CGRP), a neuropeptide released from sensory nerves, to induce oedema in orofacial tissue.
Experimental approach: Wistar rats (150–200 g) anesthetized with isoflurane were injected intraorally with CGRP (100 µl; 8–33 pmol) in the right side of the mouth. The contralateral side was injected with the same volume of physiological saline. Increased cheek thickness (in mm), as a measure of oedema formation, was assayed bilaterally with a digital caliper before ($T = 0$) and up to 24 h following injection of CGRP. Pretreatment with antagonists ($CGRP_{8–37}$, 10 nmol; pizotifen, 2 mg/kg) was given by intra-oral or subcutaneous injection, 10 or 30 min, respectively, before the inflammatory stimulus. CGRP and $CGRP_{8–37}$ were also injected into the rat hind paw to induce oedema. Data are presented as the mean (± SEM) difference in thickness between the right and the left sides at each time.
Results: Following intra-oral injection, CGRP induced a rapidly developing (5–15 min) and long-lasting (6 h), dose-dependent oedema in the rat cheek, blocked by pre-treatment with $CGRP_{8–37}$ or pizotifen. CGRP induced a smaller oedematogenic effect in the rat hind paw also blocked by the CGRP antagonist. CGRP (16 pmol) potentiated the oedema induced by co-injected substance P (3.7 nmol) and contributed to the oedema following intraoral injection of carrageenan (100 µg). Injection of $CGRP_{8–37}$ alone induced an early but short-lasting oedema.
Conclusion: Local injection of CGRP potently induced oedema in the orofacial tissue of rats which was blocked by a CGRP receptor antagonist. The overall inhibition of carrageenan-induced oedema by $CGRP_{8–37}$ suggests that endogenous CGRP contributes to an oedematogenic response in orofacial tissues.

1. Introduction

The orofacial tissues (cheeks and lips) are characterized by the presence of an extensive sensory-motor innervation and this innervation seems to be essential for the development and functioning of the orofacial organs and tissues (Pagella et al., 2014). Sensory innervation in the orofacial tissue is mainly provided by the trigeminal nerve and its branches with release of neuropeptides such as the tachykinins, substance P (SP) and neurokinin A, co-released with calcitonin-gene related peptide (CGRP; Maggi, 1995; Brain and Cox, 2006; Russell et al., 2014). Together, these neuropeptides are responsible for the so-called neurogenic inflammation, comprising reflex vasodilation, increased vascular permeability and oedema formation, in response to activation of sensory peripheral terminals (reviewed in Richardson and Vasko, 2002; Black, 2002; Kaiser and Russo, 2013; Russell et al., 2014). A range of stimuli as different as local lesions, depolarization, axonal reflexes, capsaicin or dorsal root stimulation can induce neurogenic inflammation (Richardson and Vasko, 2002; Black, 2002).

The neuropeptide SP is an important contributor to neurogenic inflammation and part of this inflammatory response is due to a secondary release of other endogenous mediators such as histamine and 5-HT from mast cells, especially in rats (Parrat and West, 1957; Eady, 1979). CGRP, co-released with SP, is a highly potent vasodilator (Brain et al., 1985) which synergises with SP to induce oedema in cutaneous tissues (Brain and Williams, 1985; Russell et al., 2014). Closer to the present context, stimulation of the trigeminal ganglion in rats increased facial blood flow mediated by CGRP (Escott et al., 1995) and the buccal mucosa of rats contains neuronal CGRP that could be released by capsaicin (Flores et al., 2001; Dussor et al., 2003).

We have recently shown that carrageenan, a well-established

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proinflammatory stimulus, injected in the oral mucosa of rats induced a distinct type of inflammatory response, characterized by the rapid formation of oedema followed by a more intense and longer-lasting response than that obtained when the same stimulus was injected in rat paws (Frade et al., 2016). Although SP injected intra-orally also induced oedema (Francischi et al., 2017), this was of much shorter duration than that induced by carrageenan (Frade et al., 2016). As CGRP is released with SP from sensory nerves and such a combination is known to show synergy in inducing oedema (Brain and Cox, 2006; Russell et al., 2014), we have assessed the oedemagenic potential of CGRP, injected locally into the orofacial tissue.

Our results showed that the orofacial tissue was responsive to the local injection of CGRP, inducing cheek oedema, and that this action of CGRP was prevented by a known antagonist of CGRP receptors, $CGRP_{8–37}$ (Chiba et al., 1989; Poyner et al., 2002). We also found that CGRP-induced cheek oedema was inhibited by pizotifen, an antagonist of 5-HT receptors and that there was only slight synergy with co-administered SP. Oedema induced by intra-oral carrageenan, although initially potentiated, was later inhibited by the CGRP antagonist. These results suggest that, in orofacial tissues, CGRP has significant oedemagenic potential by itself and that, like SP, this oedemagenic action involved the release of 5-HT. These results also support our previous findings with SP (also Frade et al., 2016; Francischi et al., 2017) that the effects of these two neuropeptides, SP and CGRP, are markedly tissue-dependent.

2. Materials and methods

2.1. Drug sources

The following drugs were used: rat calcitonin-gene related peptide (CGRP) from Genscript (lot: P15531403), Piscataway, NJ; human $CGRP_{8–37}$ (lot: 103K49521), substance P (SP) and λ-carrageenan (lot: 62H0402l) were all from Sigma-Aldrich, St Louis, MO. Pizotifen was from Sandoz (Brazil), isoflurane (Isoforine) from Cristália (Brazil), ketamine 10% (Dopalen®) and xylazine 2% (Anasedan®) were from Konig S. A (Avallaeda, Argentina). Sterile physiological saline (NaCl 0.9%) was purchased from Equiplex, Aparecida de Goiânia, GO, Brazil.

2.2. Drug preparation and administration of agonists and the CGRP antagonist

Rat CGRP, SP and λ-carrageenan (CG) were the pro-inflammatory stimuli (agonists) used in the current study and were dissolved in sterile physiological saline. Antagonists ($CGRP_{8–37}$ or pizotifen) were dissolved in physiological saline. Injections were given intraorally (CGRP, SP and CG) and or intraplantarly (CGRP) in a total volume of 100 µl in the right side of the cheek or the right hind paw, as described earlier (Frade et al., 2016). The contralateral, left, side was injected with a similar volume of saline. Control animals received an injection of saline (100 µl) in both right and left sides of the cheek. The time of saline or agonist injection was considered time zero. The antagonists ($CGRP_{8–37}$ and pizotifen) were given as a pretreatment, 10 and 30 min, intraorally or subcutaneously, respectively, before the agonist.

2.3. Animals

Male Wistar rats, weighing 150–250 g, were used throughout this study. Animals were raised and maintained at the Federal University of Minas Gerais Bioterism Center with water and food *ad libitum*, in a dark/light environment of 12/12 h and temperature control (23–26 °C). Similar environmental conditions were maintained in the laboratory during the experiments. At the end of experiments, animals were humanely killed by asphyxiation with CO_2. The study was approved by the Ethical Committee for the Use of Animals in Experimentation (Protocol numbers 97/2013 and 368/2014). Unless otherwise stated, five animals per group ($N = 5$) were used throughout the experiments. All care was taken to minimize discomfort to the animals.

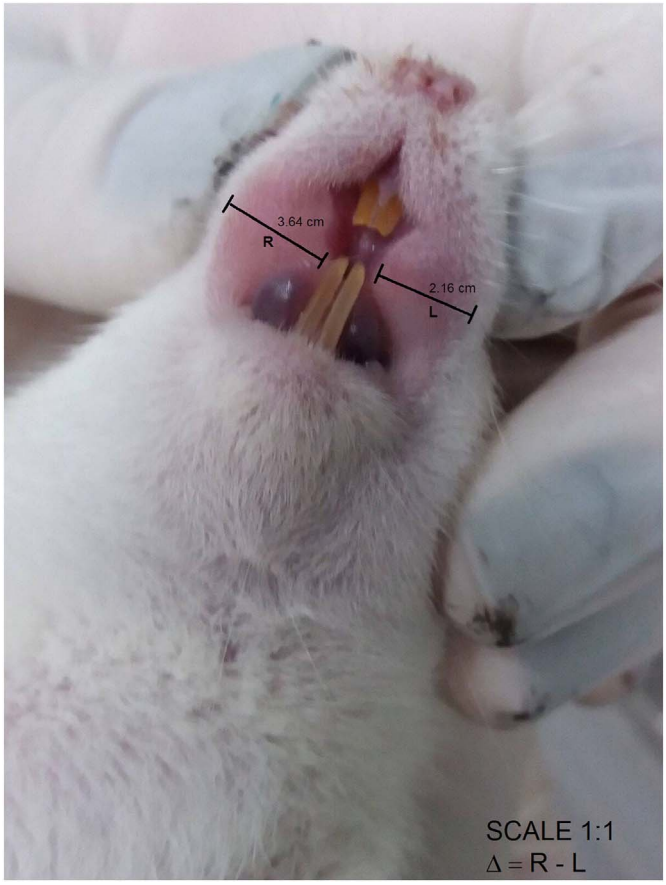

Fig. 1. Cheek measurements obtained from the indicated orofacial region. Measurements, in mm, were made with calipers. Difference (Δ) between the right and left cheeks was computed for each animal in the group to calculate the group means ± SEM.

2.4. General anesthesia

Animals were routinely anesthetized with inhaled isoflurane, as described in Francischi et al. (2017). Isoflurane was dripped onto a cotton mesh fixed in a plastic cone and the rat was gently held, nose in the cone, to breathe inside the cone. Within 30 s, the animals had lost their righting reflex and intra-oral injections could easily be made. In a separate group of rats, anesthesia was induced with a mixture of ketamine (60 mg/kg) and xylazine (15 mg/kg) given intraperitoneally (i.p.), 5 min before the intra-oral injection. Duration of anesthesia (time to recovery of the righting reflex) never exceeded 5 min after isoflurane or 1 h for the ketamine-xylazine mixture.

2.5. Cheek and paw oedema assessments

Oedema measurements were obtained essentially as described in Frade et al. (2016) and illustrated in Fig. 1. As soon as the righting reflex was lost, the initial ($t = 0$) measurement of cheek thickness (in mm) was made with digital calipers, the intra-oral injection given and then cheek thickness measured again at 5, 15 and 30 min, 1, 2, 3, 4, 6 and 24 h, following agonist or saline injections. Cheek thickness in CG-injected animals was measured at 0, 15, 30 min, 1, 2, 3, 4, 5, 6 and 24 h. Paw thickness (in mm) was obtained similarly, using the calipers at the time points indicated for cheek oedema, with the only difference being that the animals were not anesthetized for local (intraplantar) injections.

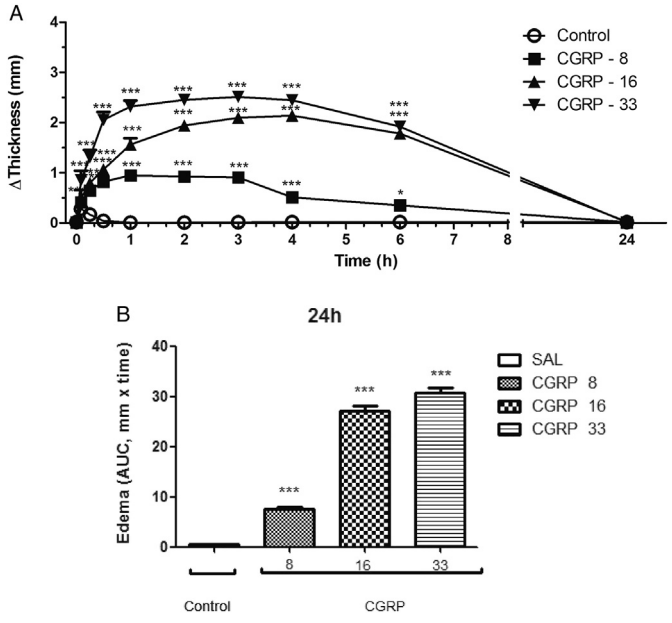

Fig. 2. CGRP induces a dose-dependent cheek oedema in rats. CGRP (8–33 pmol; 100 µl) or saline (100 µl) was injected into the right side of the submucous oral tissue (intra-oral) of rats under isoflurane anesthesia, at $t = 0$. The contralateral side was injected with the same volume of sterile saline at the same time. Control treatment (SAL) was with saline only. (A) Oedema, as the difference between thickness of right and left cheek (in mm) was measured at the times shown. (B) The area under the curve (AUC) was calculated over 24 h for the control (SAL) and each dose of CGRP. Data shown are means ± SEM; $n = 5$ per group. $^{*}P < 0.05$; $^{***}P < 0.001$: significantly different from control.

2.6. Statistics

Results are presented as the means (± SEM) of the differences in thickness between right and left side, at each time point. The area under the curve (AUC), over 6 h or 24 h, was calculated by the trapezoid rule, for each animal and the means ± (SEM) for each group of animals is shown. Statistical differences between the treatments and their respective controls were analysed by two-way ANOVA, with Bonferroni post-tests. Differences between group means were considered statistically significant when $P < 0.05$.

3. Results

The neuropeptide CGRP (8–33 pmol), after injection into the oral mucosa of rats induced a dose-and time-dependent cheek oedema, as shown in Fig. 2. The response to CGRP had a rapid onset, starting within 5 min, reaching a plateau by 1 h which was maintained until 6 h, after the injection and then slowly fading over the next 18 h. The time course of these effects over 24 h (Fig. 2A) has been transformed to $AUC_{0–24}$ values as shown in Fig. 2B. To confirm the participation of specific receptors in the cheek oedema induced by CGRP, an antagonist of CGRP receptors, $CGRP_{8–37}$ (10 nmol) was intra-orally injected, 10 min before the agonist. As shown in Fig. 3, pre-treatment with the antagonist inhibited, by about 50%, the cheek oedema induced by CGRP (16 pmol), measured over a period of 24 h (AUC_{24h}).

For comparison, we tested the oedemagenic effects of CGRP in a standard inflammatory model, the rat hind paw. The same dose of CGRP injected into the paw also induced oedema but of a lower intensity, compared with the cheek oedema (Fig. 4A). Also, in contrast to the data obtained from the cheeks, the oedema induced by CGRP in the paw had a shorter duration, lasting for < 6 h. This CGRP-induced paw oedema was markedly inhibited by the CGRP antagonist, $CGRP_{8–37}$, also given locally, before the agonist (Fig. 4B).

Because we had used anesthesia with the ketamine + xylazine mixture in our earlier work on cheek oedema (Frade et al., 2016; Francischi et al., 2017) and here we have routinely used isoflurane anesthesia, in another group of rats, we measured the effects of intraoral CGRP given under ketamine + xylazine anesthesia. As shown in Fig. 5, CGRP still induced cheek oedema under these conditions, but this response was both less intense and of shorter duration than that with isoflurane anesthesia (Fig. 2). Notably, there were no deaths during these experiments using CGRP under ketamine + xylazine anesthesia (Francischi et al., 2017).

As CGRP is often co-localised and co-released with SP in inflammatory conditions (Maggi, 1995; Brain and Cox, 2006; Russell

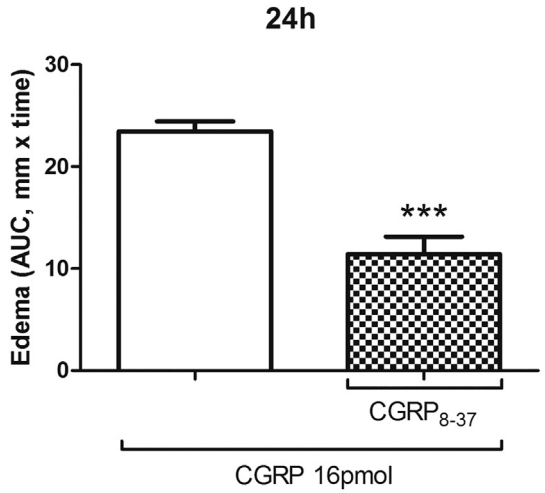

Fig. 3. Intra-oral injection of the CGRP receptor antagonist, $CGRP_{8–37}$, prevented the cheek oedema induced by CGRP. The antagonist (10 nmol; 100 µl) was injected intra-orally 10 min before the injection of CGRP (16 pmol) at $t = 0$. Measurements of cheek thickness (in mm) were made for up 24 h after CGRP. Overall oedema (as AUC 24 h) was decreased by about 50% after the pretreatment with antagonist. Data shown are means ± SEM; $n = 5$ per group. $^{*}P < 0.05$: significantly different from CGRP alone.

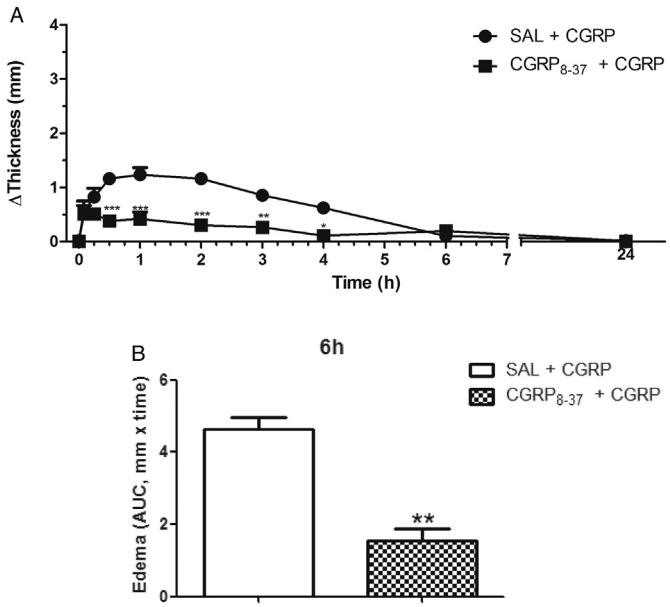

Fig. 4. Oedema induced by CGRP in rat paws was also blocked by the CGRP receptor antagonist, $CGRP_{8–37}$. CGRP (16 pmol in 0.1 ml; $t = 0$) injected 10 min after saline into the right hind paw of rats induced paw oedema with a maximum value at 1 h and lasting for < 6 h (A). Data shown are the differences between right and left (saline-treated) paws. The antagonist (10 nmol in 0.1 ml saline) was injected locally into the paw, 10 min before the injection of CGRP (16 pmol in 0.1 ml saline) and decreased both the maximum oedema and its duration, shown as a marked decrease of the AUC over 6 h (B). Data shown are means ± SEM; $n = 5$ per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$: significantly different from control.

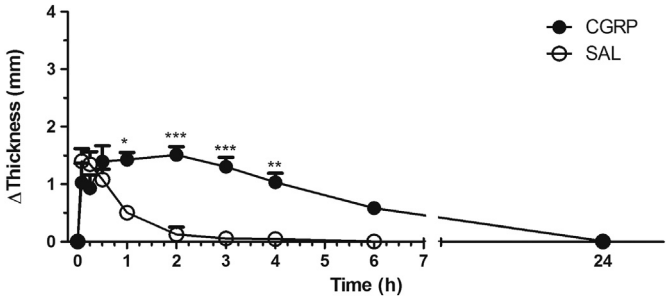

Fig. 5. A different anaesthetic (ketamine and xylazine) modifies the cheek oedema induced by intra-oral CGRP. In these experiments, anesthesia was induced by i.p. injection of ketamine (60 mg/kg) and xylazine (15 mg/kg). As soon as the righting reflexes were lost, CGRP (16 pmol; right cheek) or saline (left cheek) were injected intra-orally. Cheek thickness (in mm) was then measured at the times shown. The data show ($n = 5$ per group) that the maximal intensity and the duration of the oedema were different from the values using isoflurane anesthesia (data from Fig. 1). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$: significantly different from control (SAL).

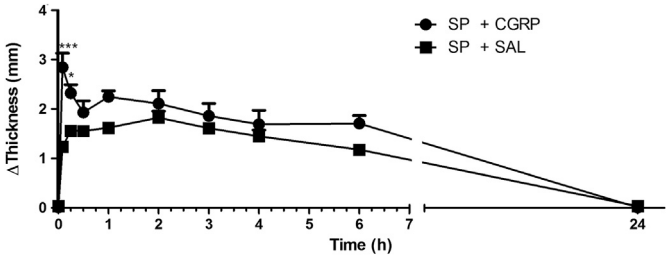

Fig. 6. Early potentiation of cheek oedema following a local injection of the combination of CGRP and SP. CGRP in saline (16 pmol in 100 µl) injected intraorally was immediately followed by an intraoral injection of SP (3.7 nmol) at time zero. Control animals were intraorally injected with SP (3.7 nmol) followed by saline (SAL). Cheek thickness (in mm) was then measured at the times shown. Data show an early potentiation of SP-induced cheek oedema by CGRP during the first hour after injection, without a change in the duration of the overall cheek oedema. Data shown are means ± SEM; $n = 5$ per group. $^{*}P < 0.05$; $^{***}P < 0.001$: significantly different from SP only (SP + SAL).

et al., 2014) and is known to synergise with SP in terms of oedema formation in rat skin (Brain and Williams, 1985), we injected a combination of CGRP (16 pmol) and SP (3.7 nmol) into rat cheeks. The results (Fig. 6) showed a potentiation of the oedema that was most marked in the early stages of the response, up to 1 h after injection. There was no significant change in the duration of the oedema. Moreover, CGRP antagonist at 10 min before didn't change the cheek oedema induced by SP (data not shown).

Another feature of SP-induced cheek oedema was its inhibition by the antagonist of 5-HT receptors, pizotifen (Francischi et al., 2017). In our present model, pretreatment with this antagonist very effectively blocked the intensity of the oedema induced by CGRP and decreased the duration of the response (Fig. 7).

One of the reasons for studying the effects of CGRP in this model was to assess the contribution of this neuropeptide to the oedema induced by a well-established inflammatory stimulus, carrageenan. This assessment was carried out with the CGRP antagonist $CGRP_{8–37}$. Pretreatment with this antagonist at a dose (10 nmol), which clearly inhibited oedema induced by CGRP itself (Fig. 3), did decrease the overall intensity of carrageenan-induced oedema in the cheek, as measured by the AUC over 6 h, without affecting its duration (Fig. 8A, B).

Unexpectedly, the antagonist actually increased the carrageenan induced oedema for the first 30 min after injection of carrageenan (Fig. 8A). This apparent "agonist" activity of $CGRP_{8–37}$ was also evident when the antagonist was given without any subsequent injection of a known oedemagenic agent (Fig. 9A). Although cheek thickness after the antagonist was significantly higher than that after saline only at 30 min, a trend towards increased thickness (=oedema) persisted for up to 6 h.

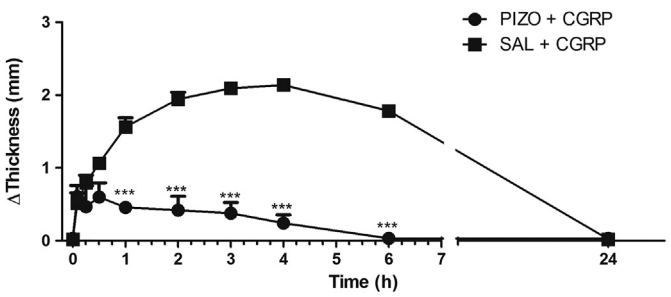

Fig. 7. Effects of pizotifen on cheek oedema induced by CGRP. Pizotifen (2 mg/kg in saline) was given by subcutaneous injection (100 µl/100 g), 30 min before the intra-oral injection of CGRP (16 pmol). Inhibition of the oedema was apparent early, about 30 min after CGRP and the maximum intensity of the oedema and its duration were markedly decreased by the pretreatment. Data shown are means ± SEM; $n = 5$ per group. $^{***}P < 0.001$: significantly different from CGRP alone (SAL + CGRP).

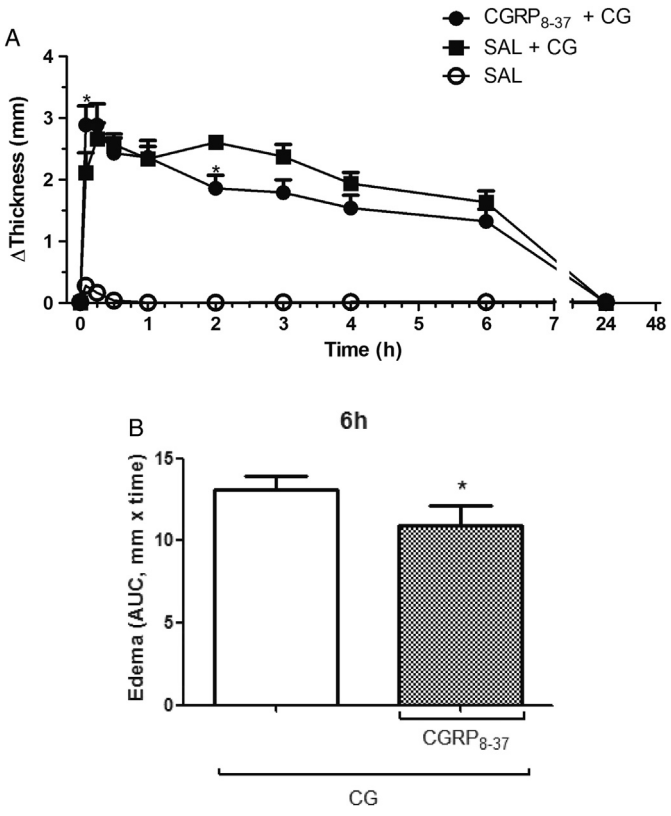

Fig. 8. Effects of $CGRP_{8–37}$ on the cheek oedema induced by intra-oral carrageenan. In (A), the oedema induced by intraoral injection of carrageenan (100 µg) showed a rapid onset and a duration of about 24 h. Pre-treatment (10 min) with the CGRP receptor antagonist $CGRP_{8–37}$ produced a very early (15 min post-injection) increase but, over 6 h, there was a significant inhibition of the oedema, by about 10%. The duration of the oedema due to carrageenan was not affected. Data shown are means ± SEM; $n = 5$ per group. $^{*}P < 0.05$; significantly different from carrageenan alone.

Expressed as AUC over 6 h, this effect of $CGRP_{8–37}$ was significantly greater than that of saline (Fig. 9B).

4. Discussion

In our experiments, we assessed the oedemagenic potential of intra-oral injections of CGRP, measuring cheek thickness as an index of oedema, a method we have developed and used in earlier work to show oedema formation in orofacial tissues following intra-oral injections of carrageenan, 5-HT, histamine and SP (Frade et al., 2016; Francischi et al., 2017). The present experiments to assess the oedemagenic potential of CGRP have produced some unexpected results.

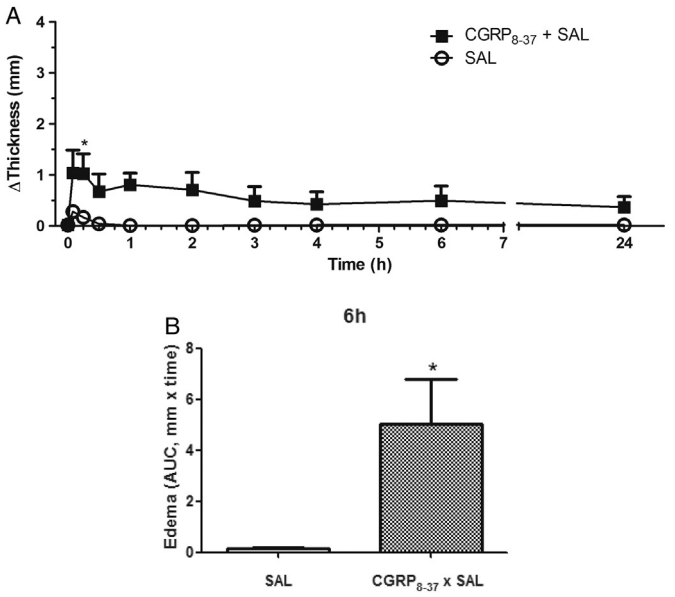

Fig. 9. A short-lived agonist activity shown by the $CGRP_{8–37}$ on cheek oedema in rats. $CGRP_{8–37}$ (10 nmol in 100 µl) was intraorally injected 10 min before saline (SAL; time = 0) and cheek oedema was measured as in Fig. 1. Contralateral cheeks were only injected with a similar volume of physiological saline. Control animals were injected only ipsilaterally with 0.1 ml of saline. A significant oedema was detected by 15 min (A) further confirmed by AUC data (B). Data shown are means ± SEM; $n = 5$ per group. $^*P < 0.05$; significantly different from control (SAL).

Our first unexpected result was that CGRP in relatively low doses (8–33 pmol), induced as much oedema as histamine, 5-HT or SP (Francischi et al., 2017), in contrast to the classical actions of this neuropeptide (Brain and Cox, 2006; Russell et al., 2014). More than 30 years ago, Brain et al. (1985) showed that CGRP was a highly potent vasodilator, relaxing rat aortic rings *in vitro* and greatly increasing blood flow in rabbit skin *in vivo*. In human skin, the vasodilation after CGRP lasted for about 5–6 h, much longer than that after histamine. Notwithstanding, a much higher dose of CGRP (250 pmol) did induce wheal and flare in human skin (Brain et al., 1985). Notably, however, the vasodilator activity of CGRP was not accompanied by increased microvascular permeability, *i.e.*, oedema, as measured by accumulation of [^{125}I]-albumin in rabbit (Brain and Williams, 1985) or rat (Brain and Williams, 1989) skin. In contrast to these findings with cutaneous blood vessels, our present results showed that CGRP by itself, did indeed induce significant levels of oedema in orofacial (cheek) tissue. We have already reported that CGRP was more potent than PGE_2 or SP in increasing vascular permeability in rat dental pulp (Maltos et al., 2004), consistent with the presence of CGRP-containing nerves and receptors (Fehrenbacher et al., 2009). Further indications of a tissue-dependent response to CGRP were provided by our data from the rat paw, where this neuropeptide again induced oedema but of less intensity and of shorter duration, than in the cheek. Taken together, our results suggest that there may be a particular response of oedema formation to CGRP in orofacial tissue, as distinct from the responses in cutaneous tissues. Nevertheless, our present results agree with the earlier findings (Brain et al., 1985), in that the effects of CGRP were longer lasting than that after SP (Francischi et al., 2017).

Our second unexpected result was the relative lack of potentiation by CGRP of the oedema induced by a low dose of SP. Our model did show some potentiation of the response to SP but that was of very short duration, about 30 min, in a response that lasted for at least 6 h. This finding contrasts with the characteristic action of CGRP in skin - its ability to synergise with various known mediators of increased microvascular permeability, including histamine, bradykinin and SP, to markedly increase oedema (Brain and Williams, 1985, 1989). Such potentiation of oedema formation was also observed after electrical stimulation of the saphenous nerve in a rat model of neurogenic inflammation (Escott and Brain, 1993), in which both SP and CGRP may be released from sensory nerves and, more recently, in human forearm skin with local perfusion of the two peptides (Schlereth et al., 2016).

A third unexpected set of results came from the effects of receptor antagonists. Whereas the inhibition of CGRP-induced oedema by pretreatment with the antagonist peptide, $CGRP_{8–37}$, confirmed the involvement of CGRP receptors in the response to intra-oral CGRP, a low level of agonist activity of $CGRP_{8–37}$ was disclosed when the antagonist was given before carrageenan and confirmed when $CGRP_{8–37}$ was given alone, *i.e.*, without any further treatment. In both conditions, there was a rapid and short-lasting increase in cheek oedema. A similar weak oedemagenic activity of the antagonist $CGRP_{8–37}$ alone, has been reported in rat skin and attributed to an indirect action *via* release of mast cell amines (Brain et al., 1992).

The effects of pizotifen, a 5-HT receptor antagonist which very effectively blocked the long-lasting oedema following CGRP imply a secondary release of 5-HT, most likely from mast cells. We tested the effects of pizotifen in the present work because we had found that SP-induced cheek oedema was blocked by this dose of pizotifen (Francischi et al., 2017). This neuropeptide (SP) has long been known to release 5-HT and histamine from rat mast cells (Fewtrell et al., 1982), but a comparable release of 5-HT in the response to CGRP is not as well documented. However, recently, using single cell analysis CGRP and SP were shown to be equally potent as releasers of 5-HT from mast cells, although the concentrations required for release were high, about 10–100 µM (Manning et al., 2016).

Our experiments assessing the effects of intra-oral CGRP using ketamine + xylazine, rather than isoflurane, as the anaesthetic were undertaken because we had found, to our dismay, that intra-oral SP with ketamine + xylazine as anaesthetic, caused a high mortality (about 60%), in contrast to SP with isoflurane with 0% mortality (Francischi et al., 2017). This high mortality appeared to be related to the stimulation of salivary glands by SP (Ekstrom et al., 1988) and by ketamine (Kohrs and Durieux, 1998). The lack of deaths in rats receiving intra-oral CGRP with ketamine + xylazine anesthesia would support our suggestion that the high mortality was due to the particular combination of SP with ketamine + xylazine anesthesia (Francischi et al., 2017). However, as observed in the present study, even in control (saline-injected) animals anesthetized with ketamine + xylazine, there was a rapid and short-lived increase in cheek thickness, suggesting that the mechanisms underlying the increased secretions associated with ketamine (Kohrs and Durieux, 1998) may also mediate this minor effect on cheek oedema formation.

Our initial goal in this study of inflammatory mediators in the orofacial tissues was, however, to understand why carrageenan, a well-established proinflammatory stimulus (Di Rosa, 1972; Vinegar et al., 1987) induced, depending on the dose studied, such a long-lasting oedema (for > 24 h) in the orofacial tissue, compared with that in the paws (Frade et al., 2016). As the duration of the oedema induced by CGRP was greater than that following several other mediators (histamine, 5-HT, SP) already tested under similar conditions (Francischi et al., 2017) and given that pretreatment with the CGRP receptor antagonist effectively reduced this response, we tested the CGRP antagonist on the oedema induced by carrageenan. We found a mixed effect of the antagonist, with initial augmentation (for 30 min) but, overall, a reduction of the oedema. The early increase could be explained by an oedemagenic effect of the antagonist, as discussed above. The later and more prolonged decrease would be compatible with the predicted antagonism of the effects of endogenous CGRP released following injection of carrageenan. Although these data indicate that CGRP and its receptors were also involved in the mediation of carrageenan-induced cheek oedema, none of the mediators tested (SP, 5HT, histamine and now CGRP) alone could account for explain the long-lasting (> 24 h) response of the orofacial tissue to intra-oral injection of carrageenan (Frade et al., 2016). Further, as both neuropeptides appear

to release, in a second stage, oedemagenic agents such as 5-HT and histamine, a closer model of the carrageenan-induced oedema might be provided by a combination of all four agents identified–SP, 5-HT, histamine and CGRP.

In summary, our data shows that the orofacial tissue is very responsive to the oedemagenic effect of the neuropeptide CGRP, an effect blocked by the known CGRP antagonist peptide $CGRP_{8–37}$, clearly demonstrating the presence of CGRP receptors in the orofacial tissue. These results along with antagonism by $CGRP_{8–37}$ of the oedema induced by carrageenan, support a role for CGRP in the inflammatory responses of the orofacial tissues. Moreover, the effects of CGRP in the orofacial tissues would also suggest that this neuropeptide, like SP, has significant tissue-dependent components to its actions.

Conflict of interest

None.

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