Chapter 12

Clonidine Inhibits the Canine External Carotid Vasodilation to Capsaicin by $\alpha_{2A/2C}$-Adrenoceptors

ABSTRACT

Migraine is a disorder associated with increased plasma concentrations of calcitonin gene-related peptide (CGRP). CGRP, a neuropeptide released from activated trigeminal sensory nerves, dilates cranial blood vessels and transmits vascular nociception. Moreover, several antimigraine drugs inhibit the dural neurogenic vasodilatation to trigeminal stimulation. Hence, this study investigated in anaesthetized dogs the effects of the α2-adrenoceptor agonist, clonidine, on the external carotid vasodilator responses to capsaicin, αCGRP and acetylcholine. 1-min intracarotid infusions of capsaicin (10, 18, 30 and 56 µg/min), αCGRP (0.1, 0.3, 1 and 3 µg/min) and acetylcholine (0.01, 0.03, 0.1 and 0.3 µg/min) produced dose-dependent increases in external carotid conductance without affecting blood pressure or heart rate. Interestingly, the carotid vasodilator responses to capsaicin, but not those to αCGRP or acetylcholine, were partially inhibited after clonidine (total dose: 24.4 µg/kg, i.v.); in contrast, equivalent volumes of saline did not affect the responses to capsaicin, αCGRP or acetylcholine. The inhibitory responses to clonidine were antagonized by i.v. administration of the α2-adrenoceptor antagonists rauwolscine (α2<sub>A/B/C</sub>; 300 µg/kg), BRL44408 (α2<sub>A</sub>; 1000 µg/kg) or MK912 (α2<sub>C</sub>; 100 and 300 µg/kg), but not by imiloxan (α2<sub>B</sub>; 1000 µg/kg). These results suggest that clonidine inhibits the external carotid vasodilator responses to capsaicin by peripheral trigeminovascular and/or central mechanisms; this inhibitory response to clonidine seems to be predominantly mediated by α2<sub>A</sub>-adrenoceptors and, to a much lesser extent, by α2<sub>C</sub>-adrenoceptors.

12.1 INTRODUCTION

Though the precise mechanisms involved in the pathophysiology of migraine remain elusive (1, 2), this neurovascular disorder has been associated with cranial vasodilatation and release of calcitonin gene-related peptide (CGRP) resulting from activation of perivascular trigeminal sensory nerves (3, 4); this cranial vasodilatation, in turn, stimulates sensory nerve transmission (5). In keeping with these findings, the antimigraine action of triptans and ergots has been attributed to: (i) a normalization of the increased levels of CGRP during migraine (6), via an inhibition of the trigeminal release of CGRP (7); and (ii) a vasoconstriction of cranial arteries (4, 8-10).

The release of CGRP can be experimentally induced by either antidromic electrical stimulation of afferent nerves (7) or chemical stimulation with capsaicin (11). In view that cranial blood vessels are innervated by CGRP-containing trigeminal sensory nerves (5) a number of strategies have attempted to induce the release of CGRP from capsaicin-sensitive trigeminal sensory nerves in experimental models of migraine (2). For example, in anaesthetized pigs, the intracarotid infusion of capsaicin induces a marked vasodilatation in the carotid circulation which is: (i) associated with an increase in plasma levels of CGRP (12); and (ii) markedly antagonized by the CGRP1 receptor antagonist, BIBN4096BS (12), at the same doses that antagonized the carotid vasodilatation to exogenous α-CGRP (13).

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>α&lt;sub&gt;A&lt;/sub&gt;</th>
<th>α&lt;sub&gt;B&lt;/sub&gt;</th>
<th>α&lt;sub&gt;C&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rauwolscine</td>
<td>9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BRL44408</td>
<td>8.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Imiloxan</td>
<td>8.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MK912</td>
<td>7.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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<td></td>
<td>5.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>6.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>8.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>9.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data taken from: <sup>a</sup>, (38); <sup>b</sup>, (50); <sup>c</sup>, (51); <sup>d</sup>, (40).
On the other hand, dihydroergotamine has been shown to induce: (i) external carotid vasoconstriction in anaesthetized dogs by stimulation of 5-HT1B receptors and α2-adrenoceptors (8); and (ii) inhibition of neurogenic vasodilatation induced by trigeminal activation, via presynaptic mechanisms (14). These findings suggest that, apart from cranial vasoconstriction, inhibition of CGRP-induced neurogenic vasodilatation may play a role in the antimigraine efficacy of some agents (14, 15). Considering the above, we decided to investigate whether selective activation of α2-adrenoceptors is capable of inhibiting the external carotid vasodilatation induced by capsaicin in an *in vivo* experimental model of migraine. Therefore, the present study in vagosympathectomized dogs set out to analyze: (i) whether clonidine, an α2-adrenoceptor agonist with antihypertensive properties (16) is capable of inhibiting the vasodilator responses to capsaicin, αCGRP and acetylcholine in the external carotid circulation; and (ii) the specific α2-adrenoceptor subtypes (α2A, α2B and/or α2C) involved in this response by investigating the effects of the α2-adrenoceptor antagonists rauwolscine (α2A/2B/2C), BRL44408 (α2A), imiloxan (α2B) or MK912 (α2C) (see Table 12.1).

### 12.2 MATERIALS AND METHODS

#### 12.2.1 General

Experiments were carried out in a total of 43 male mongrel dogs (15-20 kg) that were anaesthetised with sodium pentobarbitone (30 mg/kg, i.v.) and additional amounts (1 mg/kg, i.v.) were provided when required. All dogs were intubated with an endotracheal tube and artificially respired with room air, using a Palmer ventilator pump (20 strokes/min; stroke volume: 13-16 ml/kg) (17). Catheters were placed in: (i) the right femoral vein for the administration of vehicle, antagonists or clonidine; and (ii) the femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of arterial blood pressure. After administration of vehicle or antagonists, the venous catheter was flushed with 3 ml of saline. Mean blood pressure (MBP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures: MBP=DAP+(SAP-DAP)/3. Heart rate was measured with a tachograph (7P4F) triggered from the arterial blood pressure signal. The right common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. Thereafter, an ultrasonic flow probe (4 mm, R-series), connected to an ultrasonic T206 flowmeter (Transonic Systems Inc., Ithaca, NY, USA), was placed around this artery; thus, the flow through this bed was considered as the external carotid blood flow (18). Bilateral cervical vagosympathectomy was systematically performed in order to prevent possible baroreceptor reflexes produced by the intracarotid infusions of capsaicin, αCGRP, acetylcholine and phenylephrine. Subsequently, a catheter was introduced into the right cranial thyroid artery for the intracarotid infusions of capsaicin, αCGRP and acetylcholine. Since carotid arterioles are dilated under our experimental conditions, we had to produce a carotid preconstriction by a continuous infusion of phenylephrine (an α1-adrenoceptor agonist). For this purpose, a needle (0.5 mm diameter), connected to a catheter, was inserted into the right common carotid artery for the infusion of phenylephrine by another motor-driven syringe. This phenylephrine-induced vasoconstriction, which allows to obtain greater vasodilator responses (19), was compared to that elicited by i.v. infusions of clonidine or saline. Capsaicin, αCGRP and acetylcholine (1 ml/min for 1 min) as well as phenylephrine (0.3 ml/min continuously) were infused into the carotid artery by WPI model sp100i pumps (World Precision Instruments Inc., Sarasota, FL, USA) (for further details, see below). Arterial blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, USA). The body temperature of the animals was maintained between 37-38°C.
12.2.2 Experimental protocol

After a stable haemodynamic condition for at least 60 min, baseline values of mean blood pressure, heart rate and external carotid blood flow were determined. Then, the 43 animals were divided into three groups (n=8, 9 and 26, respectively) which were subsequently subdivided on the basis of treatment with different compounds (see Figure 12.1).

The first group (n=8) received 1-min intracarotid infusions of capsaicin (10, 18, 30 and 56 µg/min), αCGRP (0.1, 0.3, 1 and 3 µg/min) and acetylcholine (0.01, 0.03, 0.1 and 0.3 µg/min). Then, as shown in Figure 12.1, this group was subdivided into 2 subgroups that subsequently received, throughout the experiment, a continuous intracarotid infusion of, respectively: (i) vehicle (0.3 ml/min of physiological saline; n=4); and (ii) phenylephrine (6.8 µg/min; rate: 0.3 ml/min; n=4), which produces a decrease in external carotid conductance similar to that elicited by the last infusion of clonidine (see below). 20 min later, the responses to the above doses of capsaicin, αCGRP and acetylcholine were elicited again during the continuous infusion of saline or phenylephrine.

The second group (n=9) received a continuous intracarotid infusion of phenylephrine (6.8 µg/min; rate: 0.3 ml/min) and, 20 min later, the above doses of capsaicin, αCGRP and acetylcholine were analyzed. Then, as shown in Figure 12.1, this group was subdivided into 2 subgroups, so that the infusion of phenylephrine was: (i) stopped in the first subgroup (n=5); and (ii) kept continuous throughout the experiment in the second subgroup (n=4). Subsequently, by the use of another motor-driven syringe with a needle inserted into the femoral vein, the first and second subgroups received sequential continuous i.v. infusions (rate: 0.5 ml/min) of, respectively: (i) clonidine (0.01, 0.03, 0.1, 0.3 and 1 µg/kg.min; n=5) during 10 min, except the last dose which was maintained throughout the experiment in order to keep a constant external carotid vasoconstriction (similar to that by phenylephrine); and ii) equivalent volumes of physiological saline. 20 min after the start of the infusion of the last dose of clonidine or saline, the responses to the above doses of capsaicin, αCGRP and acetylcholine were elicited again (see Figure 12.1).

**Figure 12.1.** Experimental protocols followed in the 3 main groups of animals and their corresponding subdivision into different subgroups. *a*, The infusion of phenylephrine was stopped at this point; *b*, Continuous infusion of phenylephrine throughout the experiment; *c*, MK912 (100 µg/kg); *d*, MK912 (300 µg/kg).
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Figure 12.1). With this infusion scheme, clonidine reached a total dose of 24.4 µg/kg just before starting the administration of capsaicin.

Lastly, the third group (n=26) received an infusion of phenylephrine as previously described and, 20 min later, the responses to the above doses of capsaicin were elicited. Then, as shown in Figure 12.1, this group was subdivided into 6 subgroups that received i.v. bolus injections of the α₂-adrenoceptor antagonists: (i) rauwolscine (α₂A/2B/2C, 300 µg/kg; n=5); (ii) BRL44408 (α₂A, 1000 µg/kg; n=4); (iii) imiloxan (α₂B, 1000 µg/kg; n=4); (iv) MK912 (α₂C, 300 µg/kg; n=4); (v) MK912 (α₂C, 300 µg/kg; n=4); or (vi) an equivalent volume of physiological saline (0.15 ml/kg; n=5). After 15 min, each subgroup received sequential i.v. infusions of clonidine as previously described for the second group. It is important to note that after administration of rauwolscine, BRL44408 or MK912, but not of imiloxan or saline, the clonidine-induced external carotid vasoconstriction was blocked (results obtained from preliminary experiments; not shown). Therefore, in order to maintain the external carotid circulation under a vasoconstriction state similar to that before the administration of these compounds, the infusion of phenylephrine was: (i) kept continuous throughout the experiment in the subgroups receiving rauwolscine, BRL44408 or MK912; and (ii) interrupted just before the administration of imiloxan or saline (see Figure 12.1). 20 min after the start of the infusion of the last dose of clonidine (total dose: 24.4 µg/kg, as previously described), the responses to the above doses of capsaicin were elicited again.

The dose-intervals between the different doses of capsaicin, αCGRP and acetylcholine (given sequentially as they produced transient responses) ranged between 5 and 20 min, as in each case we waited until the external carotid blood flow had returned to baseline values. The doses of these compounds were selected from preliminary experiments, in which reproducible and dose-dependent increases in external carotid blood flow were elicited with no changes in blood pressure or heart rate.

12.2.3 Drugs
Apart from sodium pentobarbitone, the compounds used in this study (obtained from the sources indicated) were: capsaicin, rat αCGRP, acetylcholine chloride, clonidine hydrochloride, (l)-phenylephrine hydrochloride and rauwolscine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); BRL44408 maleate (2-[2H-(1-Methyl-1,3-dihydroisoindole)methyl]-4,5-dihydropyrimidazole maleate) and imiloxan hydrochloride (Tocris Bioscience, Ellisville, MO, USA); and MK912 ((2S-trans)-1,3,4,5',6,6',7,12b-Octahydro-1',3'-dimethyl-spiro[2H-benzofuro[2,3-a]quinoxilizine-2,4'(1'H)-pyrimidin]-2'(3'H)-one L-657,743) (gift: Dr. W.L. Henckler; Merck & Co., New Jersey, NJ, USA). The compounds were dissolved in physiological saline except capsaicin, which was dissolved in 5% v/v ethanol; this vehicle had no effect (when given by i.v. or intracarotid routes) on the systemic or carotid haemodynamic variables (not shown). The doses of all the agonists refer to their free base, whereas those of the antagonists refer to their salt. The experimental protocol of this investigation was approved by the Ethical Committee of our institution (CICUAL).

12.2.4 Data presentation and statistical analysis
All data in the text and figures are presented as mean±s.e.m. The external carotid vascular conductance was calculated by dividing blood flow (ml/min) by mean blood pressure (mmHg) and multiplied by hundred. The peak changes in external carotid conductance were expressed as percent change from baseline. The difference between the variables within one subgroup of animals was compared by using a two-way repeated measures analysis of variance (randomised block design) followed by the Student-Newman-Keuls’ test (20). Statistical significance was accepted at P<0.05 (two-tailed).
12.3 RESULTS

12.3.1 Systemic and carotid haemodynamic variables

Baseline values of heart rate, mean blood pressure and external carotid conductance in the 43 dogs were: 189±5 beats/min, 149±5 mmHg and 158±9 ml/min.mmHg, respectively. These values were not significantly modified after i.v. administration of vehicle or the antagonists BRL44408 (1000 µg/kg), imiloxan (1000 µg/kg) or MK912 (100 µg/kg) (Table 12.2). Moreover, rauwolscine (300 µg/kg) significantly increased mean blood pressure and heart rate, whilst MK912 (300 µg/kg) significantly increased heart rate (Table 12.2).

On the other hand, the continuous i.v. infusions of clonidine: (i) dose-dependently decreased the external carotid conductance, particularly during the infusion of the last three doses (155±9 ml/min.mmHg before and 130±58, 125±56, 96±43*, 70±9* and 48±5* ml/min.mmHg after, respectively 0.01, 0.03, 0.1, 0.3 and 1 µg/kg.min of clonidine; *, P<0.05); (ii) significantly decreased heart rate (184±13 beats/min before and 136±9 beats/min after 1 µg/kg.min of clonidine); and (iii) significantly increased mean blood pressure (146±12 mmHg before and 182±24 mmHg after 1 µg/kg.min of clonidine).

Moreover, the continuous intracarotid infusion of phenylephrine (6.8 µg/min; 20 min after starting the infusion) significantly decreased the external carotid conductance (165±14 ml/min.mmHg before and 55±8 ml/min.mmHg during treatment) without significant changes in mean blood pressure (159±10 mmHg before and 164±7 mmHg during treatment) or heart rate (195±7 beats/min before and 191±7 beats/min during treatment). It is to be noted that the decreased external carotid conductance during the infusion of phenylephrine (55±8 ml/min.mmHg; equivalent to an approximate decrease of 67%) did not significantly differ from that during the infusion of 1 µg/kg.min of clonidine (48±5 ml/min.mmHg; equivalent to an approximate decrease of 70%). That is why the enhanced vasodilator responses to capsaicin, αCGRP and acetylcholine during the continuous intracarotid infusion of phenylephrine were considered as the control responses when compared to those elicited during the highest infusion dose of clonidine (see below).

Table 12.2 Changes in mean blood pressure (MBP), heart rate (HR) and external carotid conductance (ECC) induced by i.v. administration of vehicle or several antagonists in anaesthetized dogs.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>MBP (mmHg)</th>
<th>HR (beats/min)</th>
<th>ECC (ml/min.mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Vehicle (0.15 ml/kg)</td>
<td>4</td>
<td>189±20</td>
<td>182±42</td>
<td>215±19</td>
</tr>
<tr>
<td>Rauwolscine (300 µg/kg)</td>
<td>5</td>
<td>149±12</td>
<td>176±14*</td>
<td>186±10</td>
</tr>
<tr>
<td>BRL444008 (1000 µg/kg)</td>
<td>4</td>
<td>161±29</td>
<td>190±25</td>
<td>193±5</td>
</tr>
<tr>
<td>Imiloxan (1000 µg/kg)</td>
<td>4</td>
<td>137±17</td>
<td>125±4</td>
<td>211±21</td>
</tr>
<tr>
<td>MK912 (100 µg/kg)</td>
<td>4</td>
<td>157±17</td>
<td>180±22</td>
<td>180±8</td>
</tr>
<tr>
<td>MK912 (300 µg/kg)</td>
<td>4</td>
<td>157±14</td>
<td>151±17</td>
<td>168±4</td>
</tr>
</tbody>
</table>

*; P<0.05 before vs. after treatment.
12.3.2 Effect of vehicle (saline), phenylephrine or clonidine on the external carotid vasodilator responses to capsaicin, αCGRP and acetylcholine

1-min intracarotid infusions of capsaicin, αCGRP and acetylcholine produced, respectively, dose-dependent increases in external carotid conductance as follows: 17±2, 23±2, 35±6 and 47±10% after 10, 18, 30 and 56 µg/min of capsaicin; 20±8, 26±11, 44±15 and 78±24% after 0.1, 0.3, 1 and 3 µg/min of αCGRP; and 4±3, 14±3, 39±12 and 57±12% after 0.01, 0.03, 0.1 and 0.3 µg/min of acetylcholine. These vasodilator responses to capsaicin, αCGRP and acetylcholine were reproducible as they remained unaffected during a continuous intracarotid or i.v. infusion of physiological saline (data not shown).

In contrast, as shown in Figure 12.2, the continuous intracarotid infusion of phenylephrine (6.8 µg/min) significantly enhanced the external carotid vasodilator responses to the 2 highest doses of capsaicin (Figure 12.2A), αCGRP (Figure 12.2B) and acetylcholine (Figure 12.2C) when compared with their

![Figure 12.2](image-url)

*P<0.05 vs. the corresponding control response.

![Figure 12.3](image-url)

*P<0.05 vs. the corresponding control response.
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respective control responses. The duration of action of the vasodilator responses to capsaicin and αCGRP (between 5 and 20 min) was longer-lasting than that to acetylcholine (between 1 and 5 min). Moreover, as shown in Figure 12.3, during the continuous intracarotid infusion of phenylephrine, the enhanced responses to capsaicin (Figure 12.3A), αCGRP (Figure 12.3B) and acetylcholine (Figure 12.3C) remained unchanged when elicited again during the continuous i.v. infusion of physiological saline. Hence, the enhanced vasodilator responses to capsaicin, αCGRP and acetylcholine during the infusion of phenylephrine were considered as the control responses when compared to those elicited during the highest infusion dose of clonidine; the latter produced a decrease in external carotid conductance similar to that by the infusion of phenylephrine (see above).

In contrast with the above, Figure 12.4 shows that the external carotid vasodilator responses to the last 2 doses of capsaicin (Figure 12.4A), but not those to αCGRP (Figure 12.4B) or acetylcholine (Figure 12.4C), were significantly – and specifically – inhibited by the continuous i.v. infusion of clonidine; the latter had reached a total dose of 24.4 µg/kg just before the administration of capsaicin (see Experimental protocol section).

12.3.3 Effect of vehicle (saline), rauwolscine, BRL44408, imiloxan or MK912 on the inhibition produced by clonidine of the capsaicin-induced vasodilator responses

Since clonidine inhibits capsaicin-induced external carotid vasodilation, the potential involvement of α2-adrenoceptors was investigated by using rauwolscine, a selective α2-adrenoceptor antagonist (see Table 12.1). Hence, Figure 12.5 shows that the inhibition by clonidine was: (i) completely antagonized by rauwolscine (300 µg/kg, i.v.; Figure 12.5B); and (ii) unaffected by an equivalent i.v. volume of saline (Figure 12.5A). Based on these findings, further experiments were carried out in order to identify the specific subtypes (α2A, α2B and/or α2C) involved in the inhibition by clonidine; for this purpose, the selective antagonists BRL44408 (α2A; 1000 µg/kg), imiloxan (α2B; 1000 µg/kg) or MK912 (α2C; 100 and 300 µg/kg) were investigated (see Table 12.1). As shown in Figure 12.6, clonidine-induced inhibition on the external carotid vasodilator responses to capsaicin was: (i) completely antagonized by BRL44408 (Figure 12.6A); (ii) partially (100 µg/kg; Figure 12.6C) or completely (300 µg/kg; Figure 12.6D) antagonized by MK912; and (iii) resistant to antagonism by imiloxan (Figure 12.6B).
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**Figure 12.5.** External carotid vasodilator responses to capsaicin before (control; during a continuous intracarotid [i.c.] infusion of phenylephrine 6.8 µg/min, 20 min later) and during a continuous i.v. infusion of clonidine (1.0 µg/kg.min, 20 min later) throughout the experiment in dogs previously administered i.v. with either: (A) vehicle (saline: 0.15 ml/kg; n=5); or (B) rauwolscine (RAUW; 300 µg/kg; n=5). *, P<0.05 vs. the corresponding control response.

**Figure 12.6.** External carotid vasodilator responses to capsaicin before (control; during a continuous intracarotid [i.c.] infusion of phenylephrine 6.8 µg/min, 20 min later) and during a continuous i.v. infusion of clonidine (1.0 µg/kg.min, 20 min later) throughout the experiment in dogs previously administered i.v. with either: (A) BRL44408 (BRL, 1000 µg/kg; n=4); (B) imiloxan (IMI, 1000 µg/kg; n=4); (C) MK912 (MK, 100 µg/kg; n=4); or (D) MK912 (MK, 300 µg/kg; n=4). *, P<0.05 vs. the corresponding control response.
12.4 DISCUSSION

12.4.1 General
Trigeminal ganglion stimulation increases cerebral blood flow associated with the release of vasoactive neuropeptides, including CGRP (21). This vasodilatation can also be produced by chemical stimulation with capsaicin in a number of vascular experimental models (11, 22, 23). Consistent with the latter findings our results show, in the first instance, that administration of capsaicin and αCGRP produced dose-dependent external carotid vasodilator responses, as previously reported in the carotid circulation of anaesthetized pigs (12, 13). Indeed, capsaicin-induced porcine carotid vasodilatation, which is associated with an increase in plasma levels of CGRP, can be antagonized by the CGRP$_1$ receptor antagonist, BIBN4096BS (12). Although it is tempting to speculate from our results that capsaicin-induced canine external carotid vasodilatation is also mediated by release of CGRP, this hypothesis is unproven by our study. Admittedly, further experiments would be required to corroborate whether capsaicin-induced canine external carotid vasodilatation is: (i) associated with an increase in plasma concentrations of CGRP; and/or (ii) amenable to blockade by a CGRP receptor antagonist (e.g. BIBN4096BS).

Irrespective of the above unproven hypothesis, our study clearly demonstrates that clonidine specifically inhibited the external carotid vasodilator responses to capsaicin, but not those to αCGRP or acetylcholine. This inhibition by clonidine, being blocked by the α$_2$-adrenoceptor antagonists rauwolscine (α$_{2A/2B/2C}$), BRL44408 (α$_{2A}$) or MK912 (α$_{2C}$), but not by imiloxan (α$_{2B}$) (see Table 12.1), is mediated by α$_2$-adrenoceptors, predominantly the α$_{2A}$ and, to a much lesser extent, the α$_{2C}$-adrenoceptor subtypes.

12.4.2 Systemic and carotid haemodynamic changes produced by the different treatments
The increase in mean blood pressure and heart rate produced by rauwolscine (Table 12.2) may be explained by blockade of presynaptic sympatho-inhibitory α$_2$-adrenoceptors, with a resulting increase in noradrenaline release (24). A similar line of reasoning could account for the increase in heart rate produced by MK912 (300 µg/kg; Table12.2), which is equipotent to rauwolscine to block α$_2A$- and α$_2C$-adrenoceptors (Table 12.1).

Moreover, in contrast to phenylephrine, the continuous i.v. infusion of clonidine, which can easily cross the blood-brain barrier (25), produced bradycardia and a vasopressor response. The clonidine-induced bradycardia, which cannot be explained by activation of a baroreceptor reflex mechanism since the animals were vagosympathectomized, may be attributed to stimulation of sympatho-inhibitory α$_2$-adrenoceptors located on: (i) cardiac sympathetic neurons; and/or (ii) the rostroventrolateral medulla. This, in turn, would result in inhibition of the sympathetic discharge (26). Furthermore, the clonidine-induced vasopressor response may be due to activation of α$_{1/2}$-adrenoceptors on vascular smooth muscle (27, 28).

On the other hand, the continuous infusions of phenylephrine (6.8 µg/min, given intracarotidly) and clonidine (1 µg/kg.min, i.v.) produced a sustained vasoconstriction in the external carotid circulation, most likely due to stimulation of, respectively, α$_1$- (29)and α$_2$- (9) adrenoceptors. It is noteworthy that phenylephrine (given intracarotidly) and clonidine (i.v.) were administered by different routes because, in preliminary experiments, we observed that clonidine given intracarotidly produced a marked external carotid vasoconstriction (a decrease of 70% in external carotid conductance) at very low doses (up to 0.5 µg/kg.min during 1 min). This low dose of clonidine did not inhibit capsaicin-induced external carotid vasodilatation probably because the plasma levels of this imidazoline were not high enough to reach (and have discernible effects in) the central nervous system. Consequently, we designed an experimental approach to produce a similar decrease in external carotid conductance (48±5 ml/min.mmHg; equivalent to an approximate decrease of 70%) by administering a continuous i.v. infusion of 1 µg/kg.min of clonidine. With this i.v. infusion scheme, clonidine reached a total dose of 24.4 µg/kg just before starting
the administration of capsaicin; this dose of clonidine (which is about 50 times higher than that given intracarotidly; see above) is high enough to reach the central nervous system.

12.4.3 Reproducibility of the canine external carotid vasodilator responses to capsaicin, αCGRP and acetylcholine
The vasodilator responses to capsaicin, αCGRP and acetylcholine did not significantly differ before and during the continuous i.v. infusion of saline, either in control dogs (lower responses; see Results section) or in dogs receiving a continuous intracarotid infusion of phenylephrine (enhanced responses; Figure 12.3); this finding indicates that the above vasodilator responses are highly reproducible. Moreover, the fact that these responses were enhanced during the continuous intracarotid infusion of phenylephrine (Figure 12.2) is attributed to the decrease in external carotid conductance resulting from an increase in the non-neurogenic vascular tone; under these conditions, there is a wider window for eliciting vasodilator responses. Since the highest dose of clonidine (1 µg/kg.min) also produced a similar decrease in external carotid conductance (see Results section), the enhanced vasodilator responses to capsaicin, αCGRP and acetylcholine during the infusion of phenylephrine were considered as the control responses when compared to those elicited during the infusion of clonidine (1 µg/kg.min). Consequently, any effect of clonidine on capsaicin-, αCGRP- or acetylcholine-induced external carotid vasodilation should be attributed to a direct interaction of this imidazoline with its respective receptors, rather than to a decrease in baseline external carotid vascular conductance.

12.4.4 Mechanisms involved in the responses to capsaicin, αCGRP and acetylcholine
The fact that capsaicin, αCGRP and acetylcholine produced dose-dependent increases in external carotid blood flow without modifying blood pressure or heart rate suggests a local vasodilator action. Regarding the mechanisms involved in these vasodilator responses, other lines of pharmacological evidence in the carotid circulation have previously shown the involvement of: (i) CGRP release and activation of BIBN4096BS-sensitive CGRP receptors for capsaicin (12); (ii) BIBN4096BS-sensitive CGRP receptors for αCGRP (13); and (iii) atropine-sensitive muscarinic receptors located on the vascular endothelium for acetylcholine (18).

12.4.5 Specific inhibition by clonidine on the vasodilator responses to capsaicin
The fact that the vasodilator responses to capsaicin, but not to αCGRP or acetylcholine, were inhibited during the infusion of clonidine (see Figure 12.4) indicates that this inhibition is: (i) specific; and (ii) unrelated to a postsynaptic interaction with CGRP receptors.

12.4.6 Possible locus of the receptors involved in the inhibitory action of clonidine
Since clonidine can cross the blood-brain barrier (25), the inhibition by clonidine in our study may involve central and/or peripheral mechanisms. This suggestion is consistent with previous studies showing that: (i) dihydroergotamine and ergotamine inhibit CGRP release after trigeminal ganglion stimulation by presynaptic mechanisms (7, 30); and (ii) UK 14,304, an α2-adrenoceptor agonist, inhibited the dural neurogenic plasma extravasation produced by trigeminal stimulation (31). Thus, we cannot categorically exclude an action of clonidine on receptors located on: (i) trigeminal vascular neurons; (ii) trigeminal ganglia; and/or (iii) trigeminal nucleus caudalis. The latter is strengthened by the fact that α2-adrenoceptors are present in trigeminal nucleus caudalis (32).

12.4.7 Role of α2-adrenoceptors in the inhibitory action of clonidine: close pharmacological resemblance to the α2A/2C subtypes
Some studies suggest that the release of substance P and CGRP from nerve endings can be inhibited by activation of presynaptic α2-adrenoceptors (31, 33). In keeping with these findings, the inhibitory action of clonidine on the vasodilatation to capsaicin was abolished by rauwolscine (Figure 12.5B) at a dose high
enough to completely – and selectively – antagonize \( \alpha_2 \)-adrenoceptors in the canine external carotid circulation (29). Although, admittedly, clonidine can also interact with imidazole binding sites (34), rauwolscine does not block these sites at concentrations that completely antagonize \( \alpha_2 \)-adrenoceptors (35, 36). Therefore, the above lines of evidence, taken together, support our contention that \( \alpha_2 \)-adrenoceptors mediate the inhibition by clonidine in our study.

\( \alpha_2 \)-adrenoceptors exist in three pharmacologically and structurally distinguishable receptor subtypes, namely \( \alpha_{2A} \), \( \alpha_{2B} \) and \( \alpha_{2C} \) (28, 37, 38). Hence, we further investigated the role of these subtypes in the above inhibition by clonidine employing antagonists with moderate to high subtype selectivity (see Table 12.1): BRL44408 (\( \alpha_{2A} \), 1000 µg/kg), imiloxan (\( \alpha_{2B} \), 1000 µg/kg) and MK912 (\( \alpha_{2C} \), 100 and 300 µg/kg) at doses high enough to completely antagonize their respective receptor subtypes in the canine external carotid circulation (39). In the first instance, it is noteworthy that the blockade produced by BRL44408 and MK912 on the inhibition by clonidine (Figure 12.6) was selective, as these compounds failed to antagonize the external carotid vasoconstriction to phenylephrine (39). Moreover, it must be pointed out that vascular \( \alpha_{2C} \)-adrenoceptors are completely antagonized by 100 µg/kg of MK912 (39), but this dose partially blocked (Figure 12.6C), whilst 300 µg/kg abolished (Figure 12.6D), the inhibition by clonidine in our study. In this respect, although MK912 displays a very high affinity for the \( \alpha_{2C} \)-subtype (pK\_i: 10.2): (i) it cannot selectively discriminate amongst the three subtypes (see Table 12.1); and (ii) no information is available on the selectivity of this subtype “selective” antagonist at canine \( \alpha_2 \)-adrenoceptor subtypes. In fact, the \textit{in vitro} \( \alpha_{2A} \)-versus \( \alpha_{2C} \)-selectivity of BRL44408 and MK912 is small (40), leaving very little room for \textit{in vivo} selectivity. These findings, coupled to the antagonism by BRL44408 (Figure 12.6A) and the inactivity of imiloxan (Figure 12.6B) on the inhibition by clonidine, lead us to suggest: (i) that mainly \( \alpha_{2A} \)-adrenoceptors and, to a much lesser extent, \( \alpha_{2C} \)-adrenoceptors are involved; and (ii) no role for \( \alpha_{2B} \)-adrenoceptors. This suggestion is in agreement with: (i) the affinity of BRL44408 and MK912 for \( \alpha_{2A} \) and \( \alpha_{2C} \)-adrenoceptor subtypes (Table 12.1); and (ii) the expression of \( \alpha_{2A} \) and \( \alpha_{2C} \)-adrenoceptors, but not \( \alpha_{2B} \)-adrenoceptors, in trigeminal ganglion neurons (41, 42) and in the trigeminal nucleus caudalis (43, 44).

12.4.8 Possible transductional mechanisms involved in \( \alpha_2 \)-adrenoceptor-induced inhibition of the vasodilator responses to capsaicin

Admittedly, our study provides no direct evidence of the transductional mechanisms involved in clonidine-induced inhibition of the vasodilator responses to capsaicin. Nevertheless, it is important to emphasize that \( \alpha_2 \)-adrenoceptors are predominantly coupled to the inhibitory heterotrimeric GTP-binding protein which: (i) inhibits the activity of adenylyl cyclase and the opening of voltage-gated Ca\(^{2+}\) channels; and (ii) activates K\(^+\) channels (45). These are signal transduction systems usually associated with a decrease in the release of neurotransmitters (28, 46, 47).

12.4.9 Possible clinical implications

It has been proposed that neurogenic dural vasodilatation (produced by an increase in the trigeminal release of CGRP) is likely to be involved in migraine pathophysiology. Hence, inhibition of this mechanism may result in antimigraine action (14, 15). Interestingly, our study shows that clonidine specifically inhibits capsaicin-induced external carotid vasodilatation (Figure 12.4), but clinical trials indicate that the antimigraine efficacy of this imidazoline does not differ from that of placebo (48). Therefore, these findings may shed further light on the mechanisms involved in the antimigraine efficacy of some agents with complex pharmacology including ergots (8, 9) and isometheptene (49). Amongst other properties, these agents activate \( \alpha_2 \)-adrenoceptors, but their antimigraine actions within the bounds of \( \alpha_2 \)-adrenoceptor activity could be mainly attributed to cranial vasoconstriction rather than inhibition of neurogenic vasodilatation.
In conclusion, the above results show that clonidine specifically inhibited the canine external carotid vasodilator responses to capsaicin. This inhibitory action of clonidine, which involves the activation of rauwolscine-sensitive \( \alpha_2 \)-adrenoceptors, seems to be predominantly mediated by \( \alpha_{2A} \)-adrenoceptors and, to a much lesser extent, by \( \alpha_{2C} \)-adrenoceptors.

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12.5 REFERENCES


