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Original article

Effect of TRPA1 activator allyl isothiocyanate (AITC) on rat dural and pial arteries

Anna Koldbro Hansted[a], Deepak Kumar Bhatt[a], Jes Olesen[a,b], Lars Jørn Jensen[c], Inger Jansen-Olesen[a,*]

[a] Department of Neurology, Danish Headache Center, Rigshospitalet, Glostrup, Denmark
[b] Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[c] Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark

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ABSTRACT

Background: Transient receptor potential ankyrin 1 (TRPA1) channels may have a role in migraine as some substances known to cause headache activate the channel. In the craniovascular system such activation causes a calcitonin gene-related peptide (CGRP)-dependent increase in meningeal blood flow. TRPA1 channels in the endothelium of cerebral arteries cause vasodilation when activated. The headache preventive substance feverfew inhibits activation of TRPA1 channels. In this study we aim to compare and characterize the effect of the TRPA1 agonist allyl isothiocyanate (AITC) on the diameter of rat dural and pial arteries *in vivo*.

Methods: The genuine closed-cranial window technique in rats was used to examine changes in dural and pial artery diameter and mean arterial blood pressure (MABP) after intracarotid infusion of AITC. Blockade experiments were performed by intravenous infusion of olcegepant, HC-030031, sumatriptan or capsazepine immediately after infusion of AITC, in four different groups of rats.

Results: AITC caused a significant dilation of dural arteries, which was inhibited by HC-030031, olcegepant and sumatriptan, but not by capsazepine.

In pial arteries AITC caused a significant dilation, which was not inhibited by any of the pre-treatments, suggesting a poor penetration of the blood-brain barrier or autoregulation due to dimethyl sulfoxide (DMSO) mediated decrease in MABP during HC-030031 infusion.

AITC did not cause a significant change in MABP.

Conclusion: AITC causes dilation of dural arteries *via* a mechanism dependent on CGRP and TRPA1 that is sensitive to sumatriptan. AITC causes a small but significant dilation of pial arteries.

Introduction

Members of the transient receptor potential (TRP) superfamily are responsible for a variety of sensory responses, including pain and temperature transduction [1]. The ankyrin subfamily has only one member, TRPA1, which is a Ca^{2+} permeable non-selective cation channel [2,3]. TRPA1 is activated by a variety of endogenous and environmental compounds such as products of oxidative stress [4], constituents of cigarette smoke [5] and components of spicy foods [6,7]. The pungent sensation experienced during ingestion of mustard is caused by the TRPA1 agonist allyl isothiocyanate (AITC), which is the main constituent of mustard oil [8]. It has been suggested that TRPA1 plays a role in headache caused by environmental irritants [9] and in migraine [10]. This is supported by the findings that umbellulone, a major constituent of the headache causing scent of the headache tree, Umbellularia californica, activates TRPA1 channels [11]. Furthermore, Feverfew (Tanacetum parthenium L.) a medicinal plant that is used for migraine prevention is known to inhibit the TRPA1 channel [12]. TRPA1 has been implicated in many different mechanisms including the sensation of noxious cold [2], vasodilation [13], inflammation [14], cough [15] and pain [16,17]. The role of TRPA1 in pain is supported by a gain-of-function mutation in the TRPA1 gene, which is associated with a pain syndrome in humans [18].

Further support of a possible role of TRPA1 in migraine is the expression of TRPA1 in dural afferent neurons [19] and the finding that activation of dural TRPA1 causes allodynia and decreased

* Corresponding author at: Department of Neurology, Forskerparken, Rigshospitalet, Nordstjernevej 42, Glostrup, 2600, Denmark
E-mail address: inger.jansen-olesen@regionh.dk (I. Jansen-Olesen).

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rearing behavior in rats [10]. Activation of TRPA1 in trigeminal neurons causes release of calcitonin gene-related peptide (CGRP) and administration of TRPA1 agonists to rats leads to a CGRP-dependent increase in meningeal blood flow [9,11]. TRPA1 was also suggested to have a role in migraine because of its expression in the endothelium of the middle cerebral artery. It is localized to myoendothelial gap junctions where activation of TRPA1 causes opening of Ca^{2+}-activated K^+ channels. This leads to a hyperpolarization of the endothelial cell, which spreads to the smooth muscle cell and causes vasodilation [20].

We hypothesize that infusion of AITC into the carotid artery causes dilation of dural arteries *via* TRPA1-dependent release of CGRP and this response is sensitive to sumatriptan treatment. Furthermore, that intra-carotid artery infusion of AITC causes dilation of pial arteries, *via* an endothelial mechanism.

Material and methods

Animals

Experiments were performed on 42 male Sprague Dawley rats (230–504 g; Taconic) under approval number 2014-15-0201-00256 from the Danish Animal Experiments Inspectorate.

Closed cranial window model

The rats were anaesthetized with an intraperitoneal injection of pentobarbital (65 mg/kg). The body temperature was maintained using a rectal thermometer and a heating pad and the trachea was cannulated and connected to a ventilator (SAR-830/P Ventilator, CWE Inc.). The femoral artery and vein were cannulated on both sides (BTPE-10, Polyethylene tubing, .011 × .024 in.(.28 × .60 mm) and BTPU-040, Polyurethane tubing, .025×.040 in.(.63 × 1.02 mm)) and secured with suture. The veins were used for infusion of anesthesia (pentobarbital 50 mg/ml; 0.15—0.23 ml/h) and drugs. The arteries were used for monitoring the mean arterial blood pressure (MABP) and collecting blood samples for blood gas analysis. A free-floating carotid catheter for drug infusion was placed in the right carotid artery using tissue glue as previously described [21]. The rat was placed in a stereotaxic frame and the right parietal bone was exposed, covered in cooled synthetic interstitial fluid and thinned to transparency using a dental drill. The cranial window was then covered with mineral oil to prevent it from drying and dural and pial arteries were viewed with an intravital microscope consisting of a Kappa CF8/5 digital camera (Kappa optronics GmbH, Gleichen, Germany) connected to a Leica Model MZ 16 microscope with a 0.5 × 10,445,929 Video Objective (Leica Microsystems, Brønshøj, Denmark). The diameter was monitored with a video dimension analyzer (V94; Living Systems Instrumentation Inc., Burlington, VT, USA). The diameter of arteries and MABP were continuously recorded and analyzed with Perisoft (Version 2.5.5; Perimed AB, Järfälla, Sweden). Before infusion of any drugs the tracheal cannula was connected to a ventilator, a blood gas analysis was made to secure that the pH, P_aCO_2 and p_aO_2 was within the normal range (pH: 7.35–7.45; P_aCO_2 35.2–42.7 mmHg; P_aCO_2: 81.7–127.5 mmHg). If needed the volume and frequency of the ventilator was adjusted within a stroke volume of 3.0–3.5 ml and a stroke rate of 50–70 per min. Another blood gas analysis was made halfway through the experiment to secure steady blood gas values.

Experimental protocol

The rats were divided in four different groups receiving AITC in combination with HC-030031, olcegepant, sumatriptan or capsazepine. All infusions were made at a rate of 125 μl/kg/min and a volume of 125 μl/kg. All experiments started with an intracarotid (*ic*) infusion of 250 μl saline followed by CGRP (500 *ng/kg, ic*) as a positive control. Then the vehicle was infused *ic* followed by two infusions of AITC (15.6 mg/kg, *ic*). Subsequently, the TRPA1 antagonist HC-030031 (3 mg/kg) [9,10], sumatriptan (600 μg/kg) (Fig. 1A), olcegepant (100 μg/kg) (Fig. 1B) or the TRPV1 antagonist capsazepine (500 μg/kg) (Fig. 1C) was infused *iv* before a third *ic* infusion of AITC. Due to the poor solubility of HC-030031 10 μl of dimethylsulfoxide (DMSO) was infused before and after the HC-030031 infusion to prevent precipitation in the catheter. As olcegepant in rat has a half-life below 1 h (e-mail correspondence

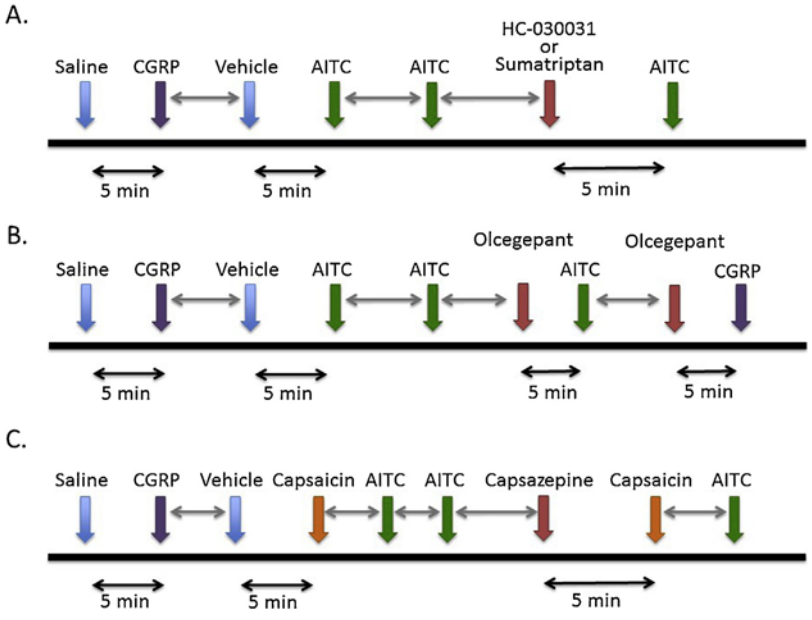

Fig. 1. Timeline of *in vivo* experiments. In all experiments saline, vehicle and antagonist were administered 5 min before drug induced effects. The grey arrow indicates that the time between these experiments vary according to the time it takes for the response to return to baseline. A. Shows the time-line for experiments with HC-030031and sumatriptan. B. Shows the time-line for experiments showing the effect of olcegepant on AITC and CGRP induced responses. C. Shows the time-line for experiments with capsazepine (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

with Dr. Klaus Rudolf, Boehringer Ingelheim) the blocker was administered twice; once before the third AITC administration and once before the second infusion of CGRP (Fig. 1B). The group receiving capsazepine also received a dose of the transient receptor potential vanilloid receptor 1 (TRPV1) agonist capsaicin (2.5 μg/kg, *ic*) before and after capsazepine (Fig. 1C).

Drugs

Allyl isothiocyanate (AITC) (Sigma-Aldrich, Denmark) and capsaicin (Sigma-Aldrich, Denmark) were diluted in 10% tween 80, 10% ethanol and 80% saline to a concentration of 125 mg/ml and 1 mg/ml, respectively. Capsaicin was further diluted with 10% tween 80, 10% ethanol and 80% saline prior to the experiment. HC-030031 (Tocris Bioscience, UK) and olcegepant (Tocris Bioscience, UK) were dissolved in 100% dimethyl sulfoxide (DMSO) to concentrations of 100 mM and 10 mg/ml, respectively. Sumatriptan succinate (Tocris Bioscience, UK) was dissolved in distilled water to a concentration of 100 mM and CGRP was dissolved in saline to a concentration of 0.5 mg/ml. Capsazepine (Tocris Bioscience, UK) was dissolved in ethanol and further diluted in 50% ethanol and 50% saline. CGRP, olcegepant and sumatriptan were further diluted with saline to their final concentration just prior to the experiment.

Data treatment and statistical analysis

The effectiveness of the test substances is based on measurements of three parameters: Changes in the diameter of dural and pial arteries and changes in MABP. The artery diameter was measured in arbitrary units and MABP in mmHg. Dilation of the arteries and changes in MABP are calculated as percentage change from the baseline, which is defined as the average of the 60 s preceding administration of test substance. Vessel diameter was measured at the peak response occurring 1 to 2 min after drug administration. Statistical analyses were made using GraphPad Prism 7 with the paired Wilcoxon non-parametric test. Groups were considered significantly different when the p-value was less than 0.05. All values are given as mean ± SEM.

Results

In the 42 experimental rats the mean blood gas values were: pH 7.42±0.008, pCO2 40.5±1.0 mmHg, pO2 112.1±3.6 mmHg. The MABP was between 89.4 and 139.9 mmHg with a mean of 117.9±7.5 mmHg.

Effect of CGRP and AITC

The effects of CGRP and AITC were both fast in onset (1–2 min) and short lasting (2–8 min).

Dural arteries

CGRP infusion to anaesthetized rats caused a significant ($p < 0.0001$) increase in dural artery diameter of 81.4±7.4% as compared to 6.0±2.8% (n = 37) after vehicle infusion (Fig. 2A). AITC caused dilation of dural arteries, which was significantly different from the vehicle response in all groups ($p < 0.05$). Combining the control AITC response from all four treatments groups (Fig. 1) the dural artery dilation was 74.8 ±11.6% (n = 32). The corresponding vehicle response was 11.6 ± 4.2%. The vehicle response was not significantly different from baseline, but significantly ($p < 0.0001$) different from the response of AITC (Fig. 2B). There was no significant difference ($p = 0.62$; n=32) between the first two administrations of AITC.

Pial arteries

Combining control data from the four treatment groups showed that intra-carotid infusion of CGRP caused a non-significant ($p = 0.121$) increase in pial artery diameter of 8.7±3.9% (n = 25) as compared to the vehicle response (−0.3±1.9%; Fig. 2C). In the same animals AITC caused a small but significant ($p = 0.0002$) increase in pial artery diameter of 21.1±5.5% as compared to −1.0±0.7% (n = 25) after vehicle infusion (Fig. 2D).

Mean arterial blood pressure

Infusion of CGRP significantly ($p < 0.0001$) decreased MABP −24.7±1.7% (n = 32) as compared to −1.3±1.7% after vehicle infusion (Fig. 2E). Infusion of AITC non-significantly ($p = 0.09$) lowered MABP by −5.5±2.5% as compared to −1.5±2.2% after vehicle (n = 30) (Fig. 2F).

Effect of capsazepine on capsaicin and AITC induced changes

In order to exclude the possibility that AITC induces its effect *via* TRPV1 receptors, we examined the ability of capsazepine to inhibit AITC induced responses. However, first the efficacy of capsazepine was tested on capsaicin-induced responses.

Dural arteries

In these experiments capsazepine reduced capsaicin-induced dilation of dural arteries from 61.8 ±7.9% to 20.9±8.4% change from baseline diameter ($p < 0.005$; n=9) (Fig. 3A).

The first two administrations of AITC caused dilation of dural arteries of 52.5±8.8% and 52.6±8.0%. Capsazepine did not reduce the dilation caused by the third AITC infusion, which was 53.9±9.8% ($p > 0.05$; n=9) (Fig. 3B).

Pial arteries

Capsaicin caused a significant dilation of 18.8±5.5% ($p = 0.016$; n=7) that was significantly reduced to 4.1±2.3% ($p = 0.047$; n=7) by capsazepine. The vehicle response in these animals was −1.5±2.9% (Fig. 3C). In the same group of animals, the response to AITC was a dilation of 45.0±16.3% ($p = 0.031$; n=7), which was non-significantly ($p = 0.109$; n=7) reduced to 23.3±6.3% after infusion of capsazepine (Fig. 3D).

Mean arterial blood pressure

Capsaicin only had a minor effect on MABP of 0.3±3.2% ($p = 0.82$; n=9). In the presence of capsazepine the capsaicin induced change from baseline was 3.5±5.4% ($p = 0.82$; n=9 when compared to capsaicin alone) (Fig. 3E). The effect of AITC on MABP was minor with a response of 0.8±4.1% before and −6.1±3.0% after capsazepine treatment. There was no significant ($p = 0.43$) effect of capsazepine treatment on the AITC induced responses (Fig. 3F).

Effect of olcegepant, HC-030031 and sumatriptan on AITC induced responses

Dural arteries

CGRP induced an increase in dural artery diameter of 93.5±15.7% ($p < 0.05$; n=7). In olcegepant treated rats the response to CGRP (26.9±9.9%) was not significantly different from saline ($p = 0.11$; n = 7); and it was significantly smaller than before olcegepant treatment ($p < 0.05$; n=7; Fig. 4A). Olcegepant significantly ($p < 0.005$; n=9) inhibited the AITC-induced diameter increase from 110.9±35.0% to 21.8±13.3% (Fig. 4B). In the group receiving treatment with the TRPA1 channel blocker HC-030031, AITC-induced dilation of dural arteries was significantly ($p < 0.01$; n=11) reduced from 65.8±13.2% to 46.0±10.8% (Fig. 4C). In the group that received sumatriptan, AITC induced dilation of dural

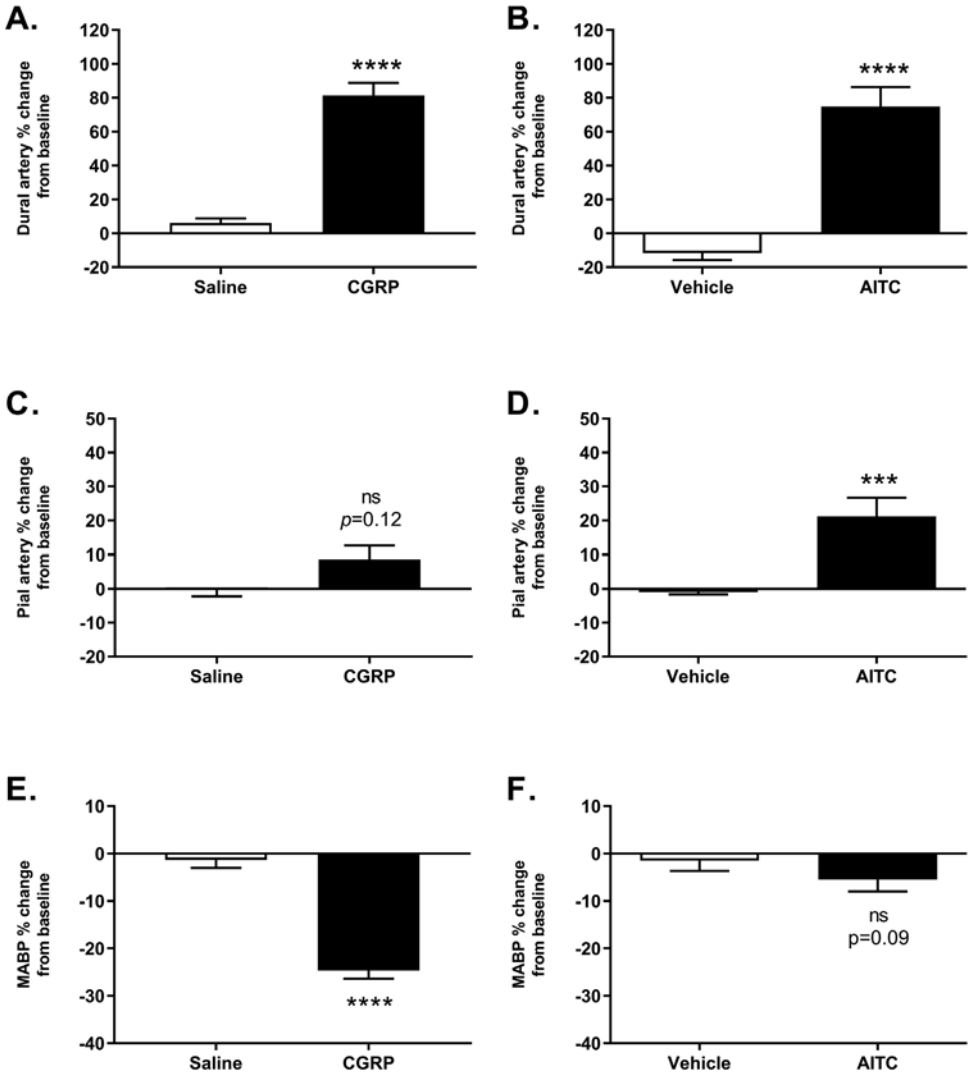

Fig. 2. Change in diameter of dural arteries (A. and B.), pial arteries (C. and D.) and of mean arterial blood pressure (MABP) (E. and F.) after infusion of CGRP (A., C. and E.) and AITC (B., D. and F.) as compared to vehicle infusion. Statistical analysis with Wilcoxon matched pairs signed rank test *** $p < 0.001$; **** $p < 0.0001$ *vs.* corresponding vehicle. The number of experiments is between 25 and 37 for CGRP and 25 and 32 for AITC.

arteries was significantly reduced ($p < 0.05$; n=6) from 66.0±19.0% to 33.9±16.4% (Fig. 4D).

Pial arteries

Pial arteries dilated in response to AITC infusion in all four groups. In the olcegepant treated group AITC caused a significant ($p < 0.05$; n=7) dilation of pial arteries of 23.0±7.0%. When AITC was administered post olcegepant infusion the dilation amounted to 16.3±8.1%. This response is not significantly different from the response seen after vehicle or AITC alone ($p > 0.05$; n=7) (Fig. 5A). In the HC-030031 treated group, AITC caused a significant ($p = 0.02$; n=8) change in pial artery diameter of 15.2±3.4% that was not inhibited ($p = 0.20$) by HC-030031. HC-030031 by itself caused a dilation of pial arteries amounting to 11.3±5.8%, which was significantly different from vehicle ($p = 0.04$; n=8). In the presence of HC-030031 AITC induced an increase in pial artery diameter of 9.4±3.3% that was not significantly different from the vehicle response of 1.2±2.2% ($p = 0.055$) (Fig. 5B).

In the sumatriptan-treated group, AITC caused a non-significant ($p = 0.09$; n=6) increase in pial artery diameter of 5.9±2.1% prior to sumatriptan infusion. Compared to vehicle the pial artery diameter reached a non-significant ($p = 0.06$; n=6) change of 9.7±3.9% when AITC was infused in the presence of sumatriptan (Fig. 5C).

Mean arterial blood pressure

In the group receiving treatment with olcegepant, AITC reduced MABP significantly ($p < 0.05$; n=7) from 2.0±1.5% after vehicle treatment to −5.5±2.9%. In the presence of olcegepant the MABP was non-significantly ($p = 0.11$; n=7) changed to -0.3±3.9% (Fig. 6A).

In the next set of experiments, we studied the effect of AITC before and after treatment with the TRPA1 channel blocker HC-030031. When given alone AITC caused a non-significant ($p = 0.76$; n=11) change in MABP of -5.3±5.8% as compared to the vehicle response of -6.4±5.7%, HC-030031 when given alone, significantly ($p < 0.05$; n=11) decreased MABP to -23.0±2.1%. The subsequent AITC administration further significantly ($p < 0.01$; n=11) decreased MABP to -25.1±4.7% (Fig. 6B).

In the sumatriptan group of rats the MABP was non-significantly ($p = 0.16$; n=6) reduced to -11.2±4.6% by the AITC infusion. After sumatriptan treatment the response to AITC was −6.5±5.1% (Fig. 6C). Interestingly, infusion of sumatriptan caused a

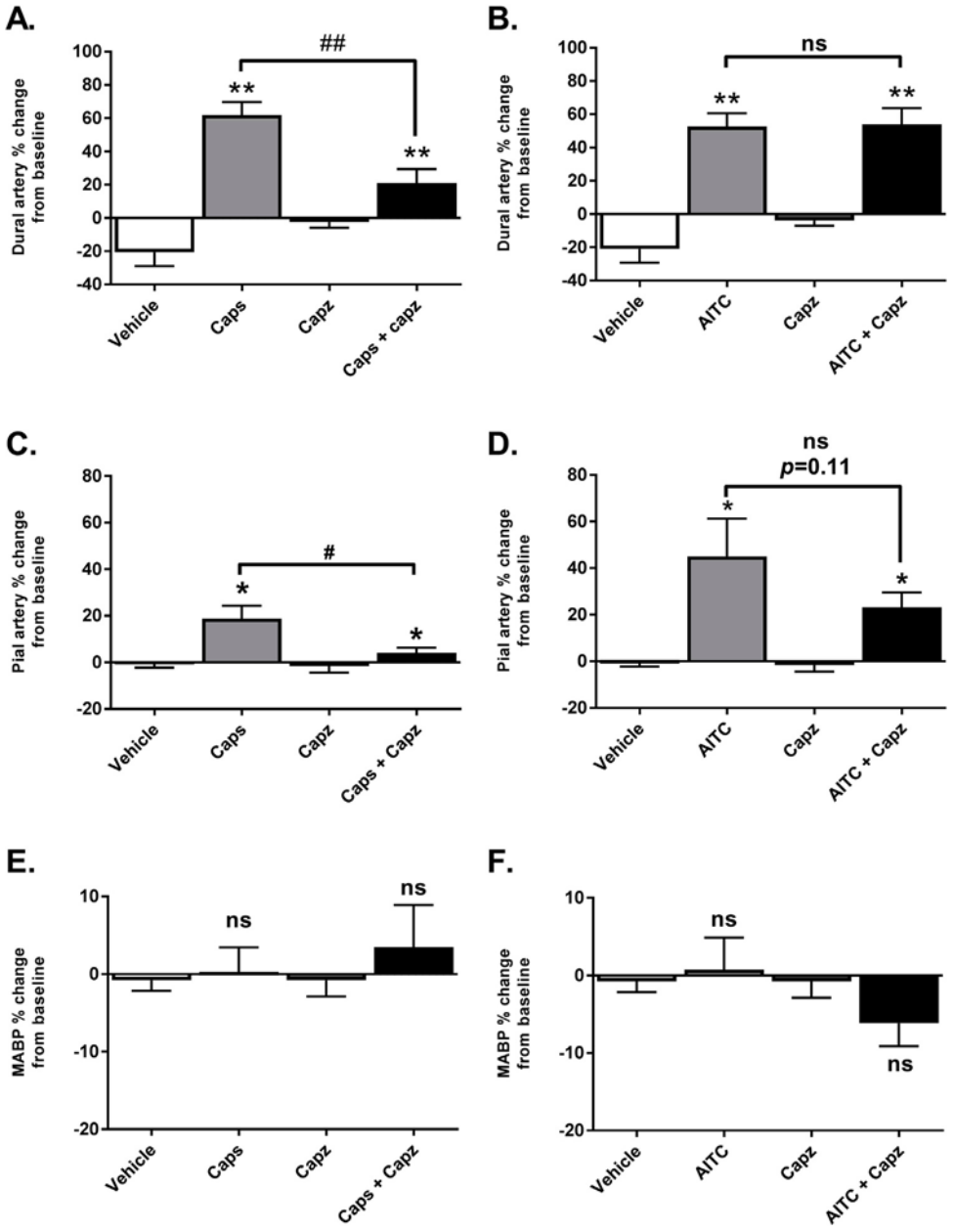

Fig. 3. Change in diameter of dural arteries (A. and B.), pial arteries (C. and D.) and of mean arterial blood pressure (MABP) (E. and F.) after infusion of capsaicin (Caps) (A., C. and E.) and Allyl isothiocyanate (AITC) (B., D.and F.) before and after treatment with the TRPV1 antagonist capsazepine (Capz). Statistical analysis with Wilcoxon matched pairs signed rank test * $p < 0.05$; ** $p < 0.01$ *vs.* corresponding vehicle; # $p < 0.05$; ## $p < 0.01$ *vs.* infusion of capsaicin without capsazepine. The number of experiments is between 7 and 9.

significant ($p < 0.05$; n=6) transient fall in MABP of −25.9±2.2% as compared to saline that was −0.03±0.7% (Fig. 6C).

HC-030031 as compared to its vehicle DMSO

HC-030031 is dissolved in DMSO, which may have an effect on the arteries and MABP. We therefore thoroughly monitored the response to DMSO when administered before the second infusion of AITC. In dural and pial arteries DMSO caused an increase of artery diameter of 5.1±12.1% and 4.8±7.4% ($p = 0.999$ and $p = 0.46$, respectively as compared to saline infusion) while the response to HC-030031 was 14.8±10.6% (n = 10) and 10.9±5.1 (n = 8), respectively. There was no significant difference between the responses to DMSO versus HC-030031 ($p = 0.91$ and $p = 0.64$, respectively) in the two arteries. Comparing the arterial responses to AITC with DMSO pre-treatment to the AITC response without DMSO pre-treatment there was no significant difference between the two provocations ($p = 0.65$ and $p > 0.999$ in dural and pial arteries, respectively).

DMSO had a significant effect on MABP amounting to −24.8±6.1% ($p = 0.006$ compared to saline infusion). HC-030031 induced a similar fall in MABP of −23.0±2.1% (n = 10; $p > 0.999$ compared to DMSO). As in the arteries, DMSO treatment did not significantly affect the response induced by AITC ($p = 0.63$).

Discussion

Several studies with TRPA1 channel activators using different routes of administration have shown an increase in meningeal blood flow *via* release of perivascular CGRP [9,11,22]. The closed cranial window technique has however, not previously been used

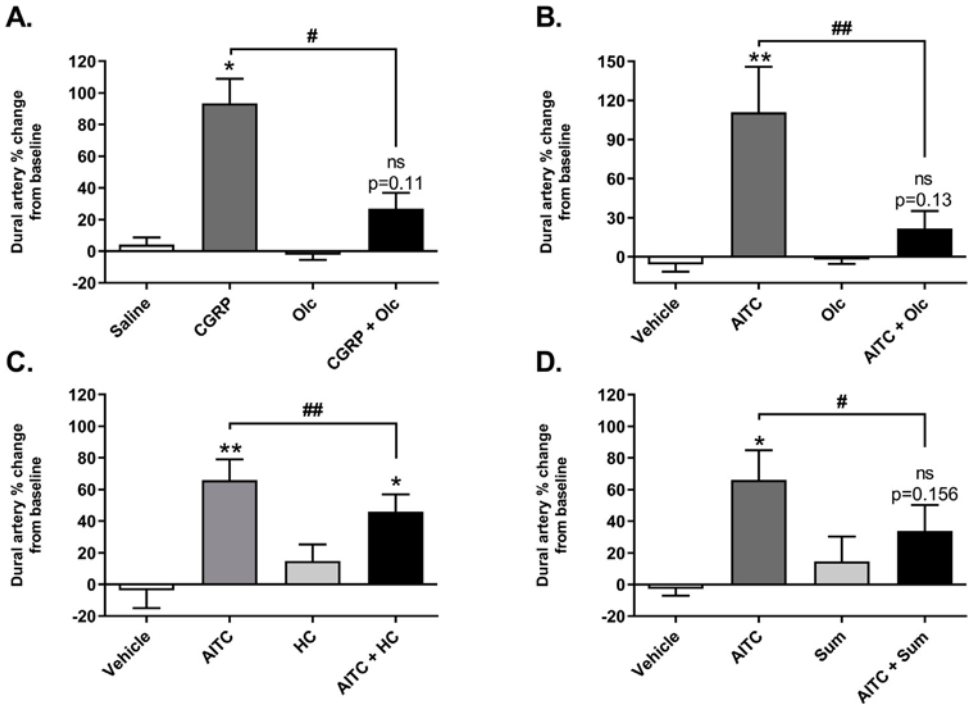

Fig. 4. Change in diameter of dural arteries after infusion of CGRP (A.) and AITC (B.) before and after treatment with the CGRP antagonist olcegepant (Olc) (A. and B.). In the lower panel the TRPA1 channel blocker HC-030031 (HC) (C.) and the 5-HT1B, 1Dand 1F agonist sumatriptan (Sum)(D.) were shown to inhibit the AITC induced increase in dural artery diameter. Statistical analysis with Wilcoxon matched pairs signed rank test * $p < 0.05$; ** $p < 0.01$ *vs.* corresponding vehicle; # $p < 0.05$ *vs.* infusion of AITC or CGRP without olcegepant or Sum; ## $p < 0.01$ *vs.* infusion of AITC without olcegepant or HC. The number of experiments is.6–11.

to study these effects on dural arteries. Using this model allows simultaneous measurement of changes in pial artery diameter and of MABP. The dual mode of TRPA1 activation of intracranial arteries (*via* the endothelium and *via* release of CGRP from perivascular nerve fibers) [11,20,22] together with the fact that dural arteries have no blood-brain barrier inspired us to simultaneously characterize the effect of TRPA1 channel agonists on pial and dural arteries. In initial pilot studies we infused the TRPA1 agonists ASP 7663 (Tocris Bioscience, UK), umbellulone (Sigma-Aldrich, US) and AITC. Throughout the experiments we were restricted by the poor water solubility of the TRPA1 selective drugs that prevented the use of higher concentrations. We found that responses could only be observed after infusion of AITC and decided to continue with this. CGRP was in all experiments used as a control of the viability of the cranial arteries. In line with previous studies using the same dose of CGRP [21], our experiments showed that infusion of CGRP caused a fall in MABP and dilation of dural arteries, while no significant changes in pial artery diameter were observed.

TRPA1 channel expression is found in a subpopulation of TRPV1 expressing neurons in rat dorsal root ganglia, where it also co-localizes with CGRP [2]. As mentioned above, AITC is a known TRPA1 agonist [6,8,23] but it has also been suggested to activate TRPV1 channels, thus contributing to its pain and visceral irritant effects [24]. However, the TRPV1 antagonist capsazepine did not inhibit any of the AITC-induced responses and thus TRPV1 channel activation seemed not to be a confounding factor in our experiments.

In line with previous studies [9–11], we found that AITC increased dural artery diameter by TRPA1 channel induced release of CGRP. An almost complete inhibition of AITC-induced dilation by olcegepant was similar to its inhibition of CGRP, suggesting that the response is entirely dependent on CGRP release.

We also found that the anti-migraine drug sumatriptan inhibits AITC induced dilation of dural arteries suggesting 5-$HT_{1B/1D/1F}$ receptor activation inhibits CGRP release *via* a mechanism shared with TRPV1 induced CGRP release. Sumatriptan was recently suggested to inhibit rearing behavior in rat induced by AITC after dural application [10].

In the cerebral circulation, TRPA1 channels are present in the endothelium causing relaxation of cerebrovascular smooth muscle cells *via* activation of small and intermediate conductance Ca^{2+} activated K^+ channels [20,26]. There were some differences in the magnitude of AITC responses between animals. However, within the same animal we had a good reproducibility between the two AITC responses that were obtained before the third AITC response, which was performed in the presence of antagonist. It may be speculated that a higher dose of AITC would activate endothelial TRPA1 channels to a more consistent magnitude in all animals. But as mentioned above, the poor water solubility of AITC did not allow us to utilize higher doses and the applied doses were high enough to induce consistent TRPA1-mediated CGRP release from dural sensory nerves. An inhibitory effect of the anesthetic agent pentobarbital on endothelium-dependent effects is another possible explanation as previously shown in rat tail arteries [27].

In the capsazepine treatment group capsaicin was administered prior to AITC. It could be speculated that capsaicin cause damage on the blood-brain barrier as suggested by Beggs and co-workers (2010). However, in their study the increase in blood-brain barrier penetration was delayed in onset and peaked at 24 h [28]. Also, it has been shown that capsaicin, at concentrations causing the release of vasoactive neuropeptides from nerve endings and increasing vascular permeability in peripheral tissues, does not increase the permeability of the blood-brain barrier [29]. In our study AITC was administered shortly after capsaicin and we have shown in pilot experiments that there is no increase in pial CGRP response when administered after capsaicin.

Pooling all AITC responses resulted in a small but significant increase in pial artery diameter without any significant effect on MABP, which suggests a direct effect of AITC on pial arteries. Olcegepant and sumatriptan did not inhibit AITC-induced pial

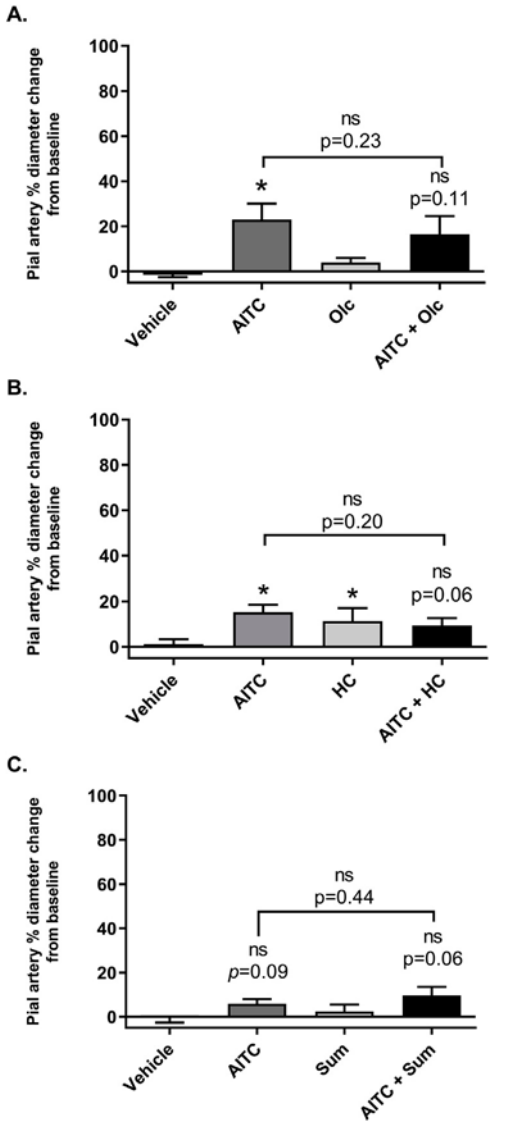

Fig. 5. Change in diameter of pial arteries after infusion of AITC before and after treatment with olcegepant (Olc) (A.) HC-030031 (HC) (B.) and sumatriptan (Sum) (C.). Statistical analysis with Wilcoxon matched pairs signed rank test * $p < 0.05$ *vs.* corresponding vehicle. The number of experiments is 6–8.

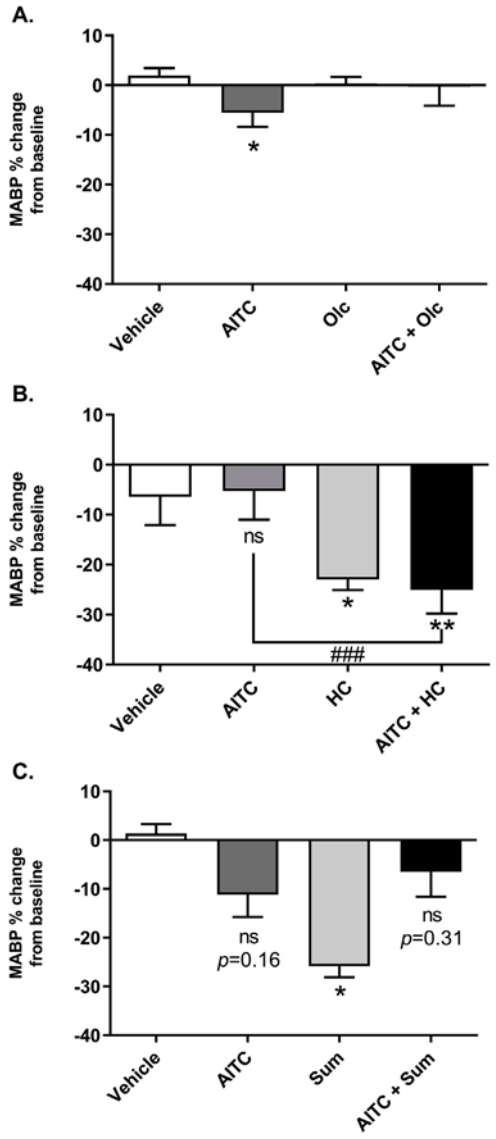

Fig. 6. Change in mean arterial blood pressure (MABP) after infusion of AITC before and after treatment with olcegepant (Olc) (A.) HC-030031 (HC) (B.) and sumatriptan (Sum) (C.). Statistical analysis with Wilcoxon matched pairs signed rank test * $p < 0.05$; ** $p < 0.01$ *vs.* corresponding vehicle. ### $p < 0.0001$ *vs.* infusion of AITC without HC-030031. The number of experiments is 6–11.

dilations suggesting the effect is not mediated *via* release of CGRP from perivascular sensory nerves. However, the TRPA1 channel antagonist HC-030031, which would inhibit the endothelial TRPA1 channels, was also ineffective. The analysis of these effects are hampered by two specific properties of pial arteries 1) the presence of the blood-brain barrier and 2) the autoregulation that protects the brain from damage during a major fall in MABP by dilation of pial arteries. To the best of our knowledge no studies have investigated the ability of AITC and HC-030031 to cross the blood–brain barrier when systemically administered. However, based on the pharmacological properties of AITC and HC-030031 (see the PubChem Public Chemical Database) both compounds may be able to penetrate the blood-brain barrier, although the extent to which this happens is not known at this time. Another possibility could be that AITC, but not HC-030031, penetrates the blood-brain barrier causing release of CGRP from sensory nerve fibers innervating pial arteries. It was found that HC-030031 caused a significant dilation of pial arteries. This dilation was of the same magnitude as when DMSO, the solvent of HC-030031, is given alone. Thus, the most probable explanation of this dilation is the simultaneous strong fall in MABP caused by DMSO with or without HC-030031 that activate brain artery autoregulation. Sumatriptan and olcegepant are water-soluble substances and cannot penetrate the blood-brain barrier. It is therefore most likely that the two substances would not influence responses originating from the cerebral side of the pial arteries.

When given alone and together with AITC, HC-030031 produced a fall in MABP of 23% and 25%, respectively. This was identical to the fall in MABP induced by its solvent DMSO. After returning to baseline DMSO did not affect the response induced by AITC. A significant transient fall in MABP was also observed during sumatriptan infusion. The effect of sumatriptan reached maximum levels from 2 to 3 minutes after its administration and returned to baseline within another 2–3 min. This is in contrast to the slight increase in MABP found in human volunteers after triptan administration that most probably is due to peripheral vasoconstriction [30,31]. However, several other studies performed in anaesthetized animals confirm our findings of a triptan-induced decrease in MABP. These studies suggested that the effect is due to

a reduction in sympathetic outflow *via* an action on central 5-HT_{1A} receptors [32,33]. Sumatriptan also activates trigeminal nociceptors, which may explain the transient fall in arterial pressure and the initial worsening of headache following sumatriptan administration [34]. Another possible mechanism is *via* the endothelium-dependent relaxation mediated by sumatriptan-induced activation of endothelial nitric oxide synthase [35].

In conclusion, AITC causes vasodilation of dural arteries *via* a mechanism that is dependent on TRPA1 channel activation leading to CGRP release that is sensitive to sumatriptan. We also suggest that AITC is a poor activator of endothelial TRPA1 channels in the genuine closed cranial window model and cannot exclude that AITC might cause a slight dilation of pial arteries after penetration of the blood-brain barrier.

Author contributions

Study Design: Inger Jansen-Olesen, Lars Jørn Jensen; Data Collection: Anna Koldbro Hansted, Deepak Kumar Bhatt; Statistical Analysis: Anna Koldbro Hansted, Deepak Kumar Bhatt and Inger Jansen-Olesen; Data Interpretation: Anna Koldbro Hansted, Inger Jansen-Olesen, Deepak Kumar Bhatt; Acceptance of final manuscript version: Inger Jansen-Olesen, Lars Jørn Jensen, Jes Olesen, Anna Koldbro Hansted, Deepak Kumar Bhatt; Literature Search: Anna Koldbro Hansted, Inger Jansen-Olesen, Lars Jørn Jensen; Funds Collection: Jes Olesen, Inger Jansen-Olesen

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Conflict of interest

There is no conflict of interest in this study.

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