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The in vivo effect of adrenomedullin on rat dural and pial arteries

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Abstract

Adrenomedullin is related to the calcitonin gene-related peptide (CGRP) family and is present in cerebral blood vessels. It may be involved in migraine mechanisms. We measured the change in dural and pial artery diameter, mean arterial blood pressure and local cerebral blood flow flux ($LCBF_{Flux}$) after intravenous (i.v.) infusion of adrenomedullin. The study was performed in the presence or absence of the CGRP1 (calcitonin-receptor-like-receptor (CALCRL)/receptor activity-modifying protein-1 (RAMP1)) receptor antagonists BIBN4096BS, CGRP-(8–37) and the adrenomedullin receptor antagonist adrenomedullin-(22–52). I.v. infusion of 15 μg kg^{-1} adrenomedullin ($n=8$) induced dilatation of dural (32 ±7.5%) and pial (18±5.5%) arteries, a reduction in mean arterial blood pressure (19±3%) and an increase in $LCBF_{Flux}$ (16±8.4%). The duration of the responses was 25 min for the dural artery, while the response of the pial artery lasted for 15 min. The CGRP1-receptor antagonists BIBN4096BS and CGRP-(8–37) and the adrenomedullin receptor antagonist adrenomedullin-(22–52) significantly inhibited the effect of adrenomedullin ($n=7$, $P<0.05$ for both arteries) on dural and pial artery diameter and mean arterial blood pressure. No significant inhibition of $LCBF_{Flux}$ was found. The antagonist alone had no effect on mean arterial blood pressure or $LCBF_{Flux}$.

In conclusion, we suggest that adrenomedullin in the rat cranial circulation dilates dural and pial arteries, reduces mean arterial blood pressure and increases $LCBF_{Flux}$, probably via a CGRP1-receptor.

Keywords: Migraine; Adrenomedullin; Adrenomedullin receptor antagonist; Cerebral blood flow; Middle cerebral artery; Middle meningeal artery

1. Introduction

Adrenomedullin consists of 50 amino acids in rat and 52 in man (Juaneda et al., 2003; Kangawa et al., 1996) and is a member of the calcitonin gene-related peptide (CGRP) family (Kangawa et al., 1996; Lang et al., 1997). Cerebral endothelial cells synthesize and secrete significant amounts of adrenomedullin (Kis et al., 2001, 2003). This results in an adrenomedullin concentration that is ~50% higher in the cerebral circulation than in the vasculature of the rest of the body (Kis et al., 2003). Adrenomedullin secreted at the abluminal membrane of the cerebral endothelial cells may enter freely into the vascular wall to cause vasodilatation and into brain interstitial fluid to directly influence neuronal function (Kis et al., 2002). In addition, adrenomedullin is synthesized in cerebral vascular smooth muscle cells and pericytes (Kis et al., 2001, 2003). Increased adrenomedullin production has been suggested to contribute to the vascular component of inflammatory disease, as the major cytokines are potent stimulators of upregulation of the adrenomedullin gene in endothelial and smooth muscle cells (Sugo et al., 1994a,b, 1995). Thus, in theory, increased concentrations of adrenomedullin could occur during neurogenic inflammation. Adrenomedullin has in addition shown to potentiate interleukin-1 (IL-1)-stimulated inducible nitric oxide synthase activity (Hattori et al., 1999). The effect of adrenomedullin is mediated through receptors expressed in smooth muscle cells and endothelial cells of cerebral vessels (Oliver et al., 2002). The receptor is a calcitonin-receptor-like-receptor (CALCRL) and for functional activity it is dependent on a receptor activity-modifying protein (RAMP) of which three types are known (RAMP1-3) (Poyner et al., 2002).

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CALCRL co-expressed with RAMP1 results in a CGRP1-receptor while CALCRL co-expressed with RAMP2 results in an adrenomedullin-receptor (Christopoulos et al., 1999; Chu et al., 2001; Poyner et al., 2002). Depending on tissue, CALCRL co-expressed with RAMP3 results in a specific adrenomedullin-receptor or a combined adrenomedullin- and CGRP-receptor (Christopoulos et al., 1999; Hay et al., 2003; Lopez and Martinez, 2002). Calcitonin gene-related peptide (CGRP) evokes marked dilatation of the cerebral and meningeal dural blood vessels (Olesen et al., 2004; Williamson et al., 1997a,b) and plays a pivotal role in migraine pathophysiology (Edvinsson, 2004).

BIBN4096BS, CGRP-(8–37) and adrenomedullin-(22–52) are CGRP/adrenomedullin receptor antagonists with a slight difference in affinity for the CALCRL/RAMP receptor complex. BIBN4096BS is a competitive and selective CALCRL/RAMP1-receptor antagonist that potently blocks the effect of CGRP in the human cranial circulation (Edvinsson et al., 2002; Verheggen et al., 2002). The antagonist is known to have higher affinity for the human CALCRL/RAMP1 combination compared to the rat CALCRL/RAMP1 (Hay et al., 2002; Poyner et al., 2002). CGRP-(8–37) produces an inhibiting effect on the CGRP-induced vasodilatation and neurogenic inflammation (Durham, 2004). However, there are controversial results concerning its specificity and potency (Brian et al., 1996; Kis et al., 2001). Adrenomedullin-(22–52) is a C-terminal fragment of human adrenomedullin (Dogan et al., 1997) known as an adrenomedullin-receptor antagonist (Hinson et al., 2000; Kis et al., 2001) with higher affinity for the CALCRL/RAMP2 receptor combination compared to the CALCRL/RAMP3 combination (Hay et al., 2003). Adrenomedullin-(22–52) has shown to inhibit in a dose-dependent manner adrenomedullin-induced cAMP formation in rat vascular smooth muscle cells (Eguchi et al., 1994).

In the present study, we aimed to investigate the hypothesis that adrenomedullin exerts effects in cranial vessels similar to those of CGRP.

2. Materials and methods

2.1. Surgical preparation

All experiments were performed in accordance with the guidelines and regulations of the Danish Animal Experimentation Inspectorate (file: 2001/561-390) on the care and use of experimental animals. The rats used in the experiments were maintained in cages with a 12-h light/dark cycle and free access to food and water. Due to hormonal variations in female rats, adult male Sprague-Dawley rats were used. They were anesthetized with pentobarbital (Mebumal® 60 mg kg^{-1} intraperitoneal) and depth of anaesthesia was tested by suppression of the hind paw reflex. Anaesthesia was continuously supplemented with pentobarbital (Mebumal® 20 mg kg^{-1} min^{-1}) i.v. during the experiment. The body temperature was maintained at 37.0±0.5 °C throughout the experiments using an automatically regulated heating blanket system (Letica® Scientific Instruments HB101, Panlab, Barcelona, Spain). Following intubation, the animal was mechanically ventilated by a respirator (Abovent, Ago Basil, Italy) with 30/70% air mixture of O_2/N_2O, a stroke volume of 3.0–3.5 ml and a stroke rate of 60–70 per min. Catheters (Portex®, Fine Bore Polythene Tubing, inner diameter 0.40 mm and outer diameter 0.80 mm, Astratech AS, Tåstrup, Denmark) were placed in the left and right femoral artery and vein for infusion of anaesthetic, test substances, measurement of mean arterial blood pressure and sampling of arterial blood for gas tension analyses. Mean arterial blood pressure was in the range of 75.8 mmHg to 134.1 mmHg, among all animals. Arterial blood samples were collected prior to, during, and at the end of the experiment, for analysis of the partial pressure of oxygen (P_aO_2), carbon dioxide (P_aCO_2) and pH (ABL520, Radiometer AS, Brønshøj, Denmark), which were kept within normal limits (pH 7.35–7.45, P_aO_2 81.7–127.5 mmHg and, P_aCO_2 35.2–42.7 mmHg).

2.2. Preparation of the closed cranial window

The animal was placed in a stereotactic frame. Skin covering the dorsal surface of the skull was retracted and the connective tissue and muscle removed, leaving the left parietal bone exposed. The bone was thinned, making a window (10×7 mm^2), by carefully drilling with a dental drill cooled by applying ice-cold isotonic saline. Drilling was continued until the middle meningeal artery and a pial artery were clearly visible through the intact skull. The cranial window was covered with mineral oil (37 °C) to avoid drying and optimise visibility and local cerebral blood flow flux ($LCBF_{Flux}$) measurements (Petersen et al., 2005a,b). After preparation of the closed cranial window a stabilisation period of at least 1 h followed before initiating the experiment.

2.3. Video microscopy and laser-Doppler flowmetry

For visualisation of the middle meningeal artery and pial artery, a video-microscope (Sony® DSP digital camera, MS 50 objective, Japan) was positioned above the window. The video camera was connected to a video dimension analyser (V94, Living Systems Instrumentation©, USA), which continuously measured the diameter of the arteries. Baseline pial and dural artery diameter was in the range of 98.1–121.1 μm and 49.1–65.4 μm, respectively. During the entire experiment, changes in dimensions arising from vessel constriction or dilatation were automatically followed by rapid time resolution and displayed on a digital panel. Two scan lines perpendicular to each artery allowed measurements of both arteries at a time in one animal. Connection of the analyser to a television monitor showed a real time image of both the middle meningeal artery and pial artery displayed on the screen. Mean arterial blood pressure and local cerebral blood flow flux ($LCBF_{Flux}$) were measured continuously and in parallel with measurements of pial and dural diameters in the same animal (Fig. 1). For the latter a laser-Doppler flowmetry (LDF) (Perimed Periflux® 4001, Perimed AB, Sweden) probe (Perimed 410, fibre separation 0.25 mm, Perimed AB, Sweden) was placed, using a micromanipulator

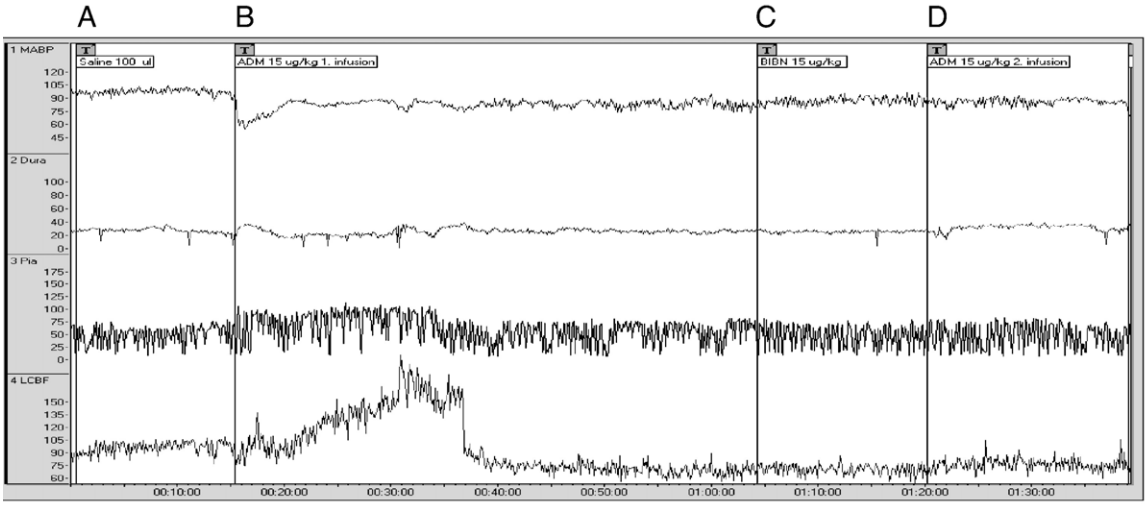

Fig. 1. Original tracing showing the effect of adrenomedullin followed by an infusion of the antagonist BIBN4096BS on dural and pial artery, mean arterial blood pressure (MABP) and local cerebral blood flow flux (LCBF): (A) administration of 100 μl saline bolus; (B) first infusion of adrenomedullin 15 μg kg^{-1} bolus; (C) infusion of BIBN4096BS 15 μg kg^{-1} bolus; (D) second administration of adrenomedullin 15 μg kg^{-1} bolus.

and a microscope, above the cortical surface of the remaining bone in touch with the mineral oil and in an area with as few vessels as possible.

All data including the changes in diameter of the arteries, mean arterial blood pressure, and $LCBF_{Flux}$ were continuously and simultaneously collected and analysed by Perisoft® (version 1; Perimed AB, Järfälla, Sweden). After the experiment, the animal was killed by an overdose of pentobarbital.

2.4. Data treatment and statistical analysis

The effectiveness of the test substances was based on measurements of four parameters: The diameter of the dural and pial artery and changes in mean arterial blood pressure and $LCBF_{Flux}$. Dural and pial vessel diameters and $LCBF_{Flux}$ were measured in arbitrary units and mean arterial blood pressure in mmHg. Dilatation of the vessels and changes in mean arterial blood pressure and $LCBF_{Flux}$ were calculated as percentage change from the baseline, which was defined as the average of the 60 s preceding the administration of test substance. Vessel diameter was measured at the peak response occurring 1 to 2 min after drug administration. All data are expressed as the mean ± S.E.M of the percentage change from baseline values with n indicating the number of rats entering the analysis. When comparing the first and second administration of adrenomedullin, a Wilcoxon's matched pair test was used and $P<0.05$ was considered statistically significant. GraphPrism® (GraphPad Software Inc., San Diego, CA, USA) was used for the statistical analysis and for the construction of graphs.

2.5. Test substances

Rat adrenomedullin and human adrenomedullin-(22–52) were obtained from Bachem AG, Bubendorf, Germany. [[*R*-(*R*,(*R*∗,*S*∗)]-*N*-[2-[[5amino-1-[[4-(4-pyridinyl)-1-piperazinyl]-carbonyl]penty]amino]-1-[3,5-dibromo-4-hydroxyphenyl)mehtyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2*H*)-quinazolinyl)-,1-Piperidinecarboxamide] code name BIBN4096BS was kindly provided by Boehringer Ingelheim Pharma KG, Biberach, Germany and rat α-CGRP-(8–37) was obtained from Neosystem, Strasbourg, France.

Rat adrenomedullin, human adrenomedullin-(22–52), and rat CGRP-(8–37) were dissolved in isotonic saline (0.9% NaCl) resulting in stock solutions of 0.105 μg μl^{-1}, 0.07 μg μl^{-1}, and 1.05 μg μl^{-1}, respectively. Afterwards the solutions were frozen at −20 °C. Prior to use, the sample was stored and diluted in isotonic saline to the desired concentration. BIBN4096BS was dissolved in a small volume (~20 μl) of 1 M hydrochloric acid, the pH adjusted to 6.75 with sodium hydroxide and diluted in isotonic saline (0.9% NaCl) to give a stock solution of 3.5 μg μl^{-1}. It was stored in frozen aliquots before use. All drugs were administered i.v. and afterwards 200 μl isotonic saline was flushed in the same catheter.

2.6. Experimental protocols

2.6.1. Dose–response relation for adrenomedullin

To find the optimal dose, 10 ($n=4$), 15 ($n=8$) and 30 ($n=4$) μg kg^{-1} (1.75, 2.62 and 5.24 nmol kg^{-1}) of adrenomedullin was given i.v. for 1 min. In each rat, the dose was administered twice to investigate reproducibility of the observed effects. It was required that the adrenomedullin dose chosen for the subsequent studies was sufficient to induce a submaximal dilatation of the dural artery with the associated decrease in mean arterial blood pressure not exceeding 25% relative to baseline pressure. First, 100 μl saline (vehicle) was administered followed 10 min later by adrenomedullin. Haemodynamic parameters were sampled at the time the baseline values were restored. The time required for returning to baseline was different for the four parameters studied. A 1-h interval between the two adrenomedullin infusions was chosen to ensure that the

effect from first adrenomedullin infusion had totally disappeared before the second adrenomedullin infusion.

2.6.2. Effect of antagonists on adrenomedullin-induced responses

About 45 min after the first adrenomedullin-infusion, 15 μg kg^{-1} BIBN4096BS (17 nmol kg^{-1}) (Petersen et al., 2004), 5 μg kg^{-1} adrenomedullin-(22–52) (1.4 nmol kg^{-1}) (Dogan et al., 1997) or 300 μg kg^{-1} CGRP-(8–37) (96 nmol kg^{-1}) (Williamson et al., 1997a,b) were administered. The second infusion of adrenomedullin was administered 15 min after BIBN4096BS or adrenomedullin-(22–52) infusion, while the time elapsed between CGRP-(8–37) and the second adrenomedullin infusion was only 2 min, as this peptide antagonist was less stable than the others.

3. Results

A total of 37 rats weighing between 300 and 350 g were included in the dose–response and agonist–antagonist studies.

3.1. Dose–response relation for adrenomedullin

In dural artery, 10 μg kg^{-1} adrenomedullin ($n=4$) induced dilatation after the first (Fig. 2) and second adrenomedullin infusions. The first administration of 10 μg kg^{-1} adrenomedullin induced a slight dilatation of pial artery diameter (Fig. 2) and an increase in $LCBF_{Flux}$ in all four rats. Repeating the infusion resulted in an increase in pial artery diameter and $LCBF_{Flux}$ in two out of four rats, while there was no response of theses two parameters in the two other rats. There was a reduction in mean arterial blood pressure after both adrenomedullin infusions (10 μg kg^{-1}). The first and second infusions of 15 μg kg^{-1} adrenomedullin ($n=8$) resulted in dilatation of the dural artery (Fig. 3). The effect lasted for 25 min. With respect to the pial artery, during the first application, two rats did not respond while in the remaining six an increase in diameter was observed after both infusions.

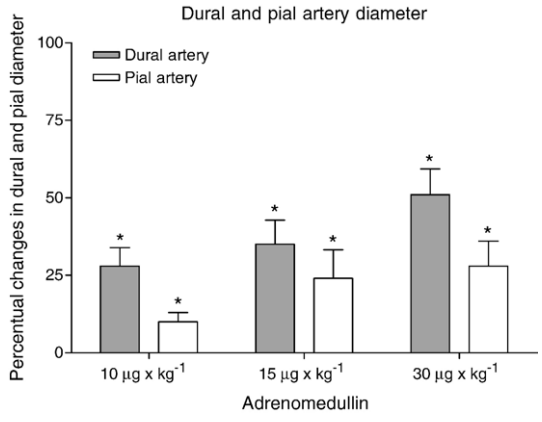

Fig. 2. Dose–response relation for adrenomedullin. Changes in percentage from baseline values of dural and pial artery diameter in response to a single administration of 10 ($n=4$), 15 ($n=8$) and 30 ($n=4$) μg kg^{-1} rat adrenomedullin. *Significantly different from baseline values ($P<0.05$).

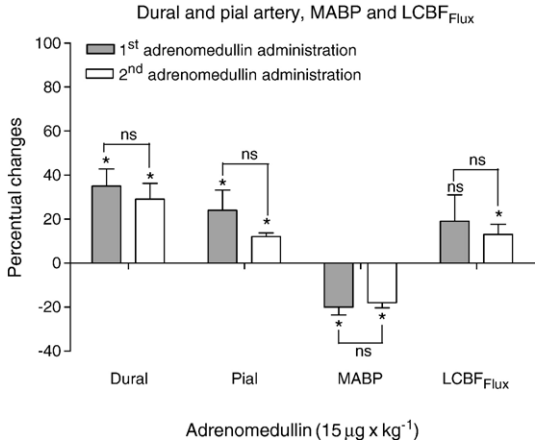

Fig. 3. Effect of adrenomedullin on dural and pial artery diameter, mean arterial blood pressure (MABP) and local cerebral blood flow ($LCBF_{Flux}$). Changes in percentage from baseline values in dural and pial artery diameter, MABP, and $LCBF_{Flux}$, during first (grey columns, control) and second (open columns) administration of 15 μg kg^{-1} adrenomedullin ($n=8$). *Significantly different from baseline ($P<0.05$). ns indicates not significantly different from baseline or from first adrenomedullin administration ($P>0.05$).

The results from the two non-responding animals are included in the average values (Fig. 3). On average, the dilatation lasted for 15 min. After first (Fig. 2) and second adrenomedullin infusion, mean arterial blood pressure was significantly reduced. The reduction lasted for 4 and 3 min, respectively. There was an increase in $LCBF_{Flux}$, which lasted for 5 min after both infusions (Fig. 3). The first infusion of 30 μg kg^{-1} adrenomedullin ($n=4$) elicited dilatation of dural and pial arteries (Fig. 2). The effect lasted for 5 min in both arteries. Mean arterial blood pressure decreased while $LCBF_{Flux}$ increased; except for in one rat in which $LCBF_{Flux}$ decreased after both adrenomedullin infusions. The 15 μg kg^{-1} adrenomedullin dose was chosen for the agonist–antagonist studies.

3.2. Effect of receptor antagonists on adrenomedullin-induced vasodilatation, mean arterial blood pressure and $LCBF_{Flux}$

Experiment on the effect of BIBN4096BS on adrenomedullin-induced vascular responses was performed in seven animals. BIBN4096BS (15 μg kg^{-1}) caused a significant inhibition of adrenomedullin-induced responses in dural and pial arteries as well as in mean arterial blood pressure. However, no significant reduction was observed in $LCBF_{Flux}$. All values are shown in Fig. 4A–D. When a single bolus of BIBN4096BS was given, no changes in any of the parameters were observed.

In the presence of adrenomedullin-(22–52) ($n=7$) (5 μg kg^{-1}) (Dogan et al., 1997), there was a significant inhibition of adrenomedullin-induced responses in dural and pial arteries as well as in mean arterial blood pressure (Fig. 4A–C). In contrast, the adrenomedullin-induced increase in $LCBF_{Flux}$ was unaffected by infusion with adrenomedullin-(22–52) ($P>0.05$) (Fig. 4D). When infused alone, adrenomedullin-(22–52) had no effect on the investigated parameters.

Following CGRP-(8–37) administration ($n=7$) (300 μg kg^{-1}) (Williamson et al., 1997a,b) the adrenomedullin-induced

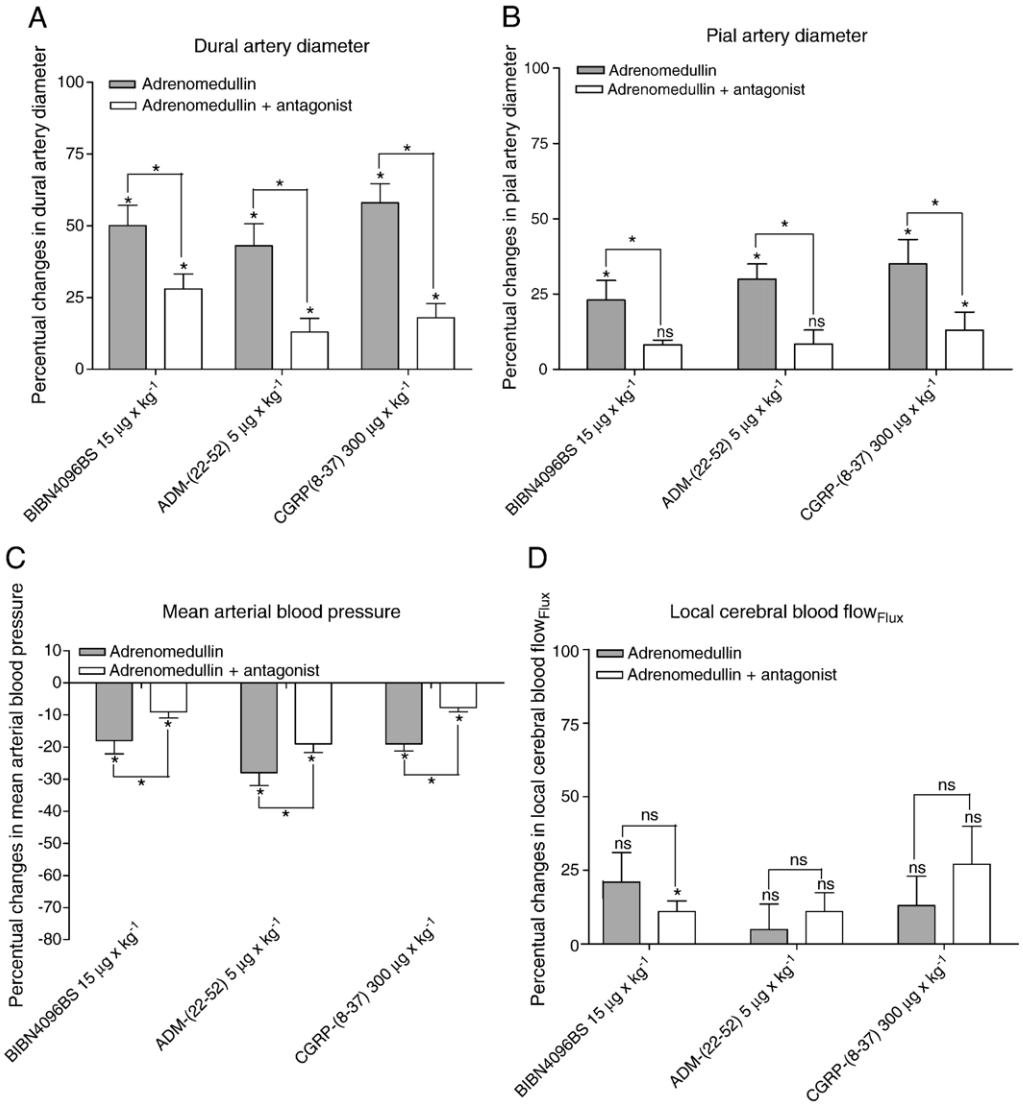

Fig. 4. (A–D) Adrenomedullin-induced changes in percentage from baseline values in (A) dural artery diameter, (B) pial artery diameter, (C) mean arterial blood pressure (MABP), and (D) local cerebral blood flow flux ($LCBF_{Flux}$) in the absence (grey columns, control) and in the presence (open columns) of the antagonists BIBN4096BS ($n=7$), human adrenomedullin-(22–52) ($n=7$) and rat CGRP-(8–37) ($n=7$), respectively. The statistical analyses compare the difference from baseline values and the difference between the two adrenomedullin administrations, respectively. *Significantly different from baseline or significantly different from first adrenomedullin administration (line between the two columns) ($P<0.05$). ns indicates not significantly different from baseline values or from first adrenomedullin administration ($P>0.05$).

dilatation of the dural and pial arteries was significantly decreased together with a significant block of the mean arterial blood pressure response to adrenomedullin (Fig. 4A–C). The $LCBF_{Flux}$ was unaffected by administration of CGRP-(8–37) (Fig. 4D).

4. Discussion

In the present study, for the first time, the effects of the vasoactive peptide adrenomedullin were investigated simultaneously in the same animal on dural and pial arteries, mean arterial blood pressure and $LCBF_{Flux}$. Additionally, the effectiveness of three CGRP-/adrenomedullin-receptor antagonists on adrenomedullin-induced responses was studied.

4.1. Dose–response relation for 10, 15 and 30 µg kg^{-1} adrenomedullin

In the initial dose–response experiment we found that 10 µg kg^{-1} elicited only a weak dilatation of the measured cranial arteries and as a consequence the dose was considered too low. In contrast, 30 µg kg^{-1} showed a strong response, but resulted in a reduction of mean arterial blood pressure. The effect on mean arterial blood pressure exceeded the maximum criteria of a 25% reduction relative to baseline pressure. However, repeated administrations of 15 µg kg^{-1} adrenomedullin resulted in minimal hypotensive effects. Furthermore, a significant and reproducible response of dural and pial arteries was obtained indicating that a second activation of the receptor was possible. We found that adrenomedullin caused a stronger

response in dural than in pial arteries. When administering drugs i.v., the existence of the blood–brain barrier (BBB) should not be underestimated. In acute experiments, adrenomedullin is unlikely to pass the BBB due to its large hydrophilic properties. However, in contrast to pial arteries, the dural arteries have no BBB and here adrenomedullin is likely to diffuse freely into the wall of the artery (Faraci et al., 1989). Thus, the small pial artery dilatation is most likely an autoregulatory response secondary to the fall in mean arterial blood pressure. This is in line with our previous studies of CGRP (Petersen et al., 2004). In the present study, $LCBF_{Flux}$ only resulted in a significant response during the second administration of 15 μg kg^{-1} adrenomedullin. This is somewhat in agreement with a previous study showing that the infusion of adrenomedullin in the rat (0.1–1.0 μg kg^{-1} min^{-1} for 60 min) increased regional CBF dose-dependently (Dogan et al., 1997).

Comparing the effects of adrenomedullin to previous studies with CGRP (Petersen et al., 2004), we found that the dose used for adrenomedullin was ~50–150 times higher than the CGRP dose required to elicit the same response indicating activation of CALCRL/RAMP1 rather than of CALCRL/RAMP2 or CALCRL/RAMP3. This supports previous data proposing that CALCRL/RAMP1 is dominant when both RAMP1 and RAMP2 are present in a cell (Buhlmann et al., 1999). Regarding desensitization of the adrenomedullin receptor, previous studies have described conflicting information (Hay and Smith, 2001). In our pilot study, the effect of adrenomedullin lasted 25 min. Therefore, in the present study a 1-h interval between first and second adrenomedullin infusion was chosen to avoid carry-over effects.

4.2. Effect of antagonists on adrenomedullin responses in dural and pial arteries

Our present study shows that the three antagonists were effective in inhibiting the vasoactive effect of adrenomedullin in vivo. We used a concentration of BIBN4096BS that has been shown to cause a ~50% maximum inhibition of CGRP-induced responses in our in vivo model (Petersen et al., 2004). BIBN4096BS has a high affinity for the human cerebral CGRP1-receptor in vitro (Edvinsson et al., 2002) and the antagonist has shown to antagonize the CGRP-infused extracerebral effect in humans (Petersen et al., 2005a,b). BIBN4096BS has not shown any antagonist activity for the adrenomedullin-receptor (Hay et al., 2003). Therefore, the effect of adrenomedullin is most possibly mediated primarily through activation of the CGRP1-receptor, which supports previous findings (Lang et al., 1997).

The best available antagonist for the adrenomedullin-receptor, adrenomedullin-(22–52), has become established as a weak and non-specific antagonist (Hinson et al., 2000; Kis et al., 2001). However, the recently demonstrated preference of adrenomedullin-(22–52) to bind to the CALCRL/RAMP2 combination before the CALCRL/RAMP3 combination could explain large variations between different tissues (Hay et al., 2003). In the present study adrenomedullin-(22–52) significantly blocked the effects on both dural and pial arteries (the autoregulatory effect due to changes in mean arterial blood pressure still being kept in mind). This is in accordance with a previous study where the effects of adrenomedullin in cerebral circulation was blocked by adrenomedullin-(22–52) (Dogan et al., 1997). Whether this response is due to an effect on CGRP- or adrenomedullin-receptors is difficult to interpret since the existence of other receptor-subtypes is possible. However, due to the preference of adrenomedullin-(22–52) to the adrenomedullin-receptor, the presence of a specific adrenomedullin-receptor, additional to the CGRP-receptor, is strengthened.

The characteristics of the CGRP1-receptor antagonist CGRP-(8–37), is controversial. Some authors consider it specific and potent (Brian et al., 1996), while others find it unspecific and non-potent (Hinson et al., 2000; Kis et al., 2001). We found, which is in agreement with previous reports (Lang et al., 1997), that CGRP-(8–37) significantly inhibited the adrenomedullin-induced vasodilatory effects ($P<0.05$).

4.3. Effect of adrenomedullin and antagonists on mean arterial blood pressure and $LCBF_{Flux}$

In all experiments first administration of adrenomedullin induced a slight increase in $LCBF_{Flux}$ that was not significantly different from baseline values. This is in accordance with a previous study performed in anesthetized rats (Dogan et al., 1997). When a single bolus of the antagonists was given, no change in mean arterial blood pressure and $LCBF_{Flux}$ was observed. The present study demonstrates that BIBN4096BS significantly attenuated the adrenomedullin-induced decrease in mean arterial blood pressure while no significant attenuation on $LCBF_{Flux}$ was observed. Similar results were found after administration of adrenomedullin-(22–52) and CGRP-(8–37). The most likely explanation of our results is that $LCBF_{Flux}$ is partly autoregulated in the present model.

In conclusion, adrenomedullin evokes dilatation of cranial arteries in a fashion similar to CGRP but less potently. Notably, BIBN4096BS, adrenomedullin-(22–52) and CGRP-(8–37) were effective in inhibiting the adrenomedullin-induced dilatation of both dural and pial arteries as well as attenuation of the decrease in mean arterial blood pressure while the effects on $LCBF_{Flux}$ were less clear. Based on these novel observations in rat, we conclude that adrenomedullin exerts effect on dural arteries while the effect on pial arteries most probably is due to autoregulation. This effect of adrenomedullin is, however, less potent than CGRP and is mediated via CGRP1-receptors rather than adrenomedullin-receptors.

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