No effect of the angiotensin receptor blocker candesartan on cerebrovascular autoregulation in rats during very high and low sodium intake

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Abstract

Autoregulation of cerebral blood flow (CBF) denotes that CBF is constant despite fluctuation of blood pressure within wide limits. Inhibition of the renin–angiotensin system (RAS) is known to decrease the lower and upper limits of CBF autoregulation. We have previously shown that this includes inhibition by the angiotensin receptor blocker (ARB) candesartan. In the present study we investigated the influence of the ARB candesartan on the lower limit of CBF autoregulation in two groups of Sprague-Dawley rats, on high (4.0% Na$^+$) and low (0.004% Na$^+$) sodium diet, respectively. Control animals were given the same diet, but no ARB. CBF was studied with the laser Doppler method. Blood pressure was lowered by controlled bleeding. Results revealed that both high and low sodium diet with low and high renin levels respectively block the influence of candesartan on CBF autoregulation. This was expected in rats on a high salt diet with a low renin level, but unexpected in rats with a low salt intake with a high renin level.

Introduction

Cerebral blood flow (CBF) is autoregulated, i.e. kept constant during fluctuations in systemic blood pressure within wide limits. If blood pressure falls below the lower limit of autoregulation, CBF decreases, and if blood pressure rises above the upper limit of autoregulation, CBF increases.[1,2] The renin–angiotensin system (RAS) exerts a tone in the resistance vessels of the brain. Inhibition of this system with either an angiotensin converting enzyme inhibitor (ACEi) or an angiotensin receptor blocker (ARB) shifts the lower and upper limits of autoregulation of CBF towards lower blood pressure levels. This has been shown with the ACEis captopril, ceranopril, fosinopril, and enalapril,[3–5] and the ARBs candesartan and valsartan,[5–7] that block the angiotensin II subtype 1 (AT1) receptor. We found that a single dose of candesartan caused a significant shift of the lower limit of the order of 7 mmHg in Sprague-Dawley rats fed standard chow with a sodium content of 0.25%.[5]

Plasma renin activity (PRA) is influenced by changes in the sodium content of the diet, high sodium intake resulting in low PRA and low sodium intake in high PRA.[8] In a previous study our group has shown that the effect the ACEi captopril on the lower limit of CBF autoregulation is not dependent on the presence of circulating renin.[9] Further we have shown that the ARB candesartan had the same effect on CBF autoregulation as the ACEi captopril.[5] The aim of the present study was to investigate the influence of the

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ARB candesartan on the lower limit of CBF autoregulation when manipulating the renin activity by changes in sodium intake. It was hypothesized that a low salt intake/high renin activity would conserve or even amplify the effect of candesartan blocking the effect of renin; while a high salt intake/low renin activity might suppress or even abolish the effect.

Methods

Studies of CBF autoregulation were carried out in male Sprague-Dawley rats, weight about 250 g, corresponding to an age of 50 days, obtained from Charles River, Germany. All procedures were conducted in accordance with the FELASA guidelines for animal research and approved by The Danish Animal Experiments Inspectorate (license number 2007/561-1320) as well as by The Department of Experimental Medicine at The Faculty of Health and Medical Sciences, the University of Copenhagen. The study is reported in accordance with ARRIVE guidelines.

Groups of animals, chow, and medication

The animals were divided into two main groups: (1) a group fed high sodium diet (4.0% Na^+); (2) a group fed low sodium diet (0.004% Na^+). The diets were given for 7 days and were supplied by Brogaarden, Denmark and Zeigler, USA, respectively. All the animals had unlimited access to drinking water. Both main groups were further divided into a control group and a group that was given a single dose of the ARB candesartan (AstraZeneca, Sweden) 0.2 mg/kg intravenously as an infusion lasting two min. This is the same dose as in our previous study on CBF autoregulation.[5] Thirty-two animals were investigated, eight in each group. To prevent clotting of the catheters all animals were given 200 IE of heparin intravenously.

An additional group of six animals on normal salt intake only received candesartan 0.2 mg/kg and blood pressure was followed for 2 h. This group was studied only to demonstrate a biological effect of candesartan. No other procedures were performed and only blood pressure was measured.

Surgical procedures

All animals were studied under general anesthesia with isoflurane (Baxter, USA) and N_2O. During induction the dose of isoflurane was 5%, while it was set to 2.5% during surgery and 1.7% during CBF measurements. No muscle relaxant was given. A tube was surgically placed in the trachea for mechanical ventilation. Polyethylene catheters were inserted in both femoral arteries and both femoral veins. One arterial catheter was used for monitoring blood pressure and the other for arterial blood sampling to monitor pO_2 and pCO_2. One of the venous catheters was used for drug infusion. After the insertion of the catheters the

animal was placed in a stereotactic apparatus. With a small dental drill a craniotomy was done and care was taken not to damage the dura that was kept intact. A laser Doppler probe was placed onto the dura away from the larger vessels, to measure CBF.

Measurements and data analysis

After induction of anesthesia and before surgical intervention, venous blood was sampled from a tail vein (60 μL) and analyzed for K^+, Na^+, urea, and creatinine by standard clinical chemistry methods at the department of clinical chemistry at our hospital, and for analysis of plasma renin concentrations (PRCs).

PRC was measured by the rate of generation of Angiotensin I in the presence of excess angiotensinogen by the antibody trapping method,[10] modified as previously described by one of the authors.[11] Angiotensinogen excess was obtained by addition of plasma from nephrectomized sheep. Results are reported as milli international units per liter (mIU/L) of the activity of the standard WHO renin sample (National Institute for Biological Standards and Control, Hertfordshire, UK) measured along with the plasma samples in each assay.

Baseline blood pressure was recorded in all animals following anesthesia and placement of catheters in the femoral arteries and veins, but before craniotomy and medication with norepinephrine and candesartan.

To prevent an initial fall in blood pressure below the lower limit of the autoregulation of CBF during and after craniotomy, norepinephrine 0.5–9 μg/min was infused intravenously keeping mean arterial blood pressure (MABP) stable at about 85–100 mmHg. Intravenous norepinephrine will not influence CBF.[2] Thereafter an intravenous injection of candesartan (lasting 2 min) was given. During the following 20 min blood pressure stabilized at a lower level. Then the experiment continued with sequentially reducing and finally discontinuing the norepinephrine infusion, followed by controlled stepwise bleeding to lower the blood pressure (Figure 1). A waiting period of about 2 min ensued after every reduction in norepinephrine infusion and later between the controlled bleeding that was between 0.1 and 0.2 mL at a time to make certain that a new steady state in both MABP and CBF was achieved. The whole procedure lowering the blood pressure took about 45 min. Arterial blood samples were collected to measure pO_2 and pCO_2 at the start and at the end of the experiment. Initial hemoglobin levels were measured in the first arterial blood sample. The respirator was adjusted as necessary to maintain stable pCO_2. Data were collected on a PC running MS Windows 98 SE running Perisoft 2.5 via Periflux 5000 and a PF 472 A/D Converter Box from Perimed AB, Sweden.

The laser Doppler flowmetry has been validated at our laboratory for estimating the lower limit of CBF autoregulation,[12] and has been used in several disease models.[13–15] The
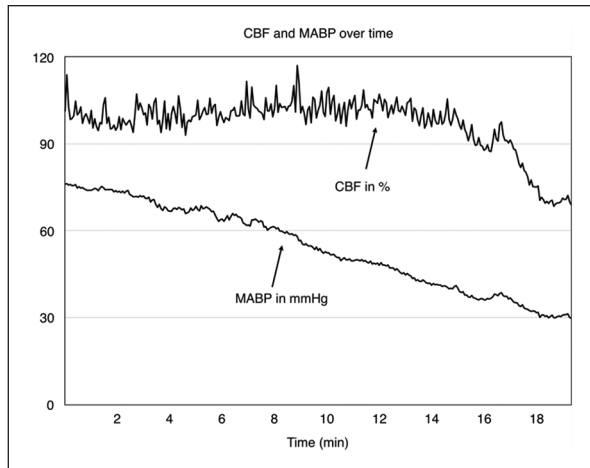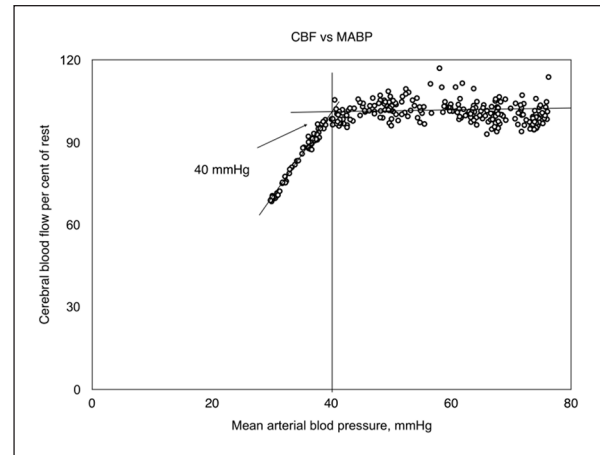
Figure 1. Time course of mean blood pressure (MABP) and cerebral blood flow (CBF) illustrated in a single animal.

Figure 2. The lower limit of autoregulation of cerebral blood flow. All simultaneous measurement of CBF and blood pressure are shown from a single animal study. Two slopes are calculated one for the points below and one for those above a chosen point among the measurements. Calculating for all measurement points, the slopes yielding the best fit (lowest sum of square) are used, and the lower limit of autoregulation is defined as the intersection between the two slopes.

equipment continuously monitored local cortical cerebral perfusion by multiple measurements (Figure 1). From the laser Doppler signal a relative value for CBF is calculated and expressed as per cent of baseline. The method does not give absolute CBF values, but relative flow in arbitrary units.

The lower limit is defined as the intersection between a slope regression line which represents the measured levels clearly below the lower limit and a plateau regression line which in turn represents the measurements above the lower limit. The lines are found by including additional measurements in the slope until a best fit is found, defined as the least sum of squares of the deviations from the different sets of lines. The method of calculation represents a modification of the method described in detail in a previously published paper.[9] In that paper a horizontal line was used to fit the plateau, but here we allowed this line to have a slope (Figure 2). Constraints on the intersection point were used on the slope as described previously,[9] but their significance is minor in the present study which included multiple measuring points and thereby better-defined slopes. The program used to calculate the lower limit was a DOS program that was run on a MacBook Pro running OS X 10.6.2 and emulating DOS using DOSBox v.0.73. Statistical analysis was done on a MacBook Pro running OS X 10.6.2 using Prism 5.0c. Data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to compare the four groups to each other. A t-test was used to compare variables between the high sodium and low sodium groups, and to compare variables between control and candesartan within each group.

Results

There was no difference among the animal groups in pCO_2 and pO_2 levels during CBF measurements. Data for arterial blood gases and lower limits of CBF autoregulation are shown in Tables 1 and 2. Biochemical variables are

shown in Table 3. Animals on low sodium diet had raised PRC compared to the animals on high sodium diet. In the low sodium animals, Na^+ levels were lower compared to the animals on high sodium diet whereas urea, creatinine and hemoglobin levels were raised. K^+ levels were not statistically different. Data for biochemical variables are shown in Table 3. The animals in the high sodium group gained more weight and MABP was lower in the high sodium group after induction of anesthesia compared to the animals on low sodium diet. The high sodium animals consumed water in much greater amounts than the animals in the low sodium group and urinary output was correspondingly larger (not measured quantitatively). Data for weight and blood pressure are shown in Table 4.

There was no significant difference between the lower limit of autoregulation in the four groups of rats where CBF was monitored, and especially no tendency to a lower limit following candesartan in neither the high nor the low salt intake groups. Both high sodium and low sodium diets thus appeared to block the effect of candesartan on the lower limit found in an earlier study.[6]

In the additional group of animals only receiving one dose of candesartan identical for all animals, a clear-cut biological effect was demonstrated. MABP dropped from about 90 to about 50 mmHg following candesartan and remained at that level for 2 h (Figure 3).

Discussion

The finding that there was no difference in the lower limit of CBF autoregulation during blockade of the AT1 receptor in any of the four animal groups was not predicted, as

Table 1. Arterial blood gases.

	High sodium		Low sodium	
	Control mean ± SD N = 8	Candesartan mean ± SD N = 8	Control mean ± SD N = 8	Candesartan mean ± SD N = 8
start pCO_2 (kPa)	4.7 ± 0.20	5.1 ± 0.20	4.9 ± 0.21	4.9 ± 0.46
end pCO_2 (kPa)	5.0 ± 0.22	5.1 ± 0.26	4.9 ± 0.21	5.1 ± 0.37
start pO_2 (kPa)	19.9 ± 5.22	19.2 ± 2.91	20.3 ± 2.90	18.3 ± 1.23
end pO_2 (kPa)	20.3 ± 5.81	21.1 ± 2.98	23.0 ± 2.69	21.3 ± 2.29

"Start" and "end" refer to values at the beginning and the end of the measurement of the autoregulatory curve.
No significant differences with multiple comparison (ANOVA) test.

Table 2. Lower limit of CBF autoregulation.

	High sodium		Low sodium	
	Control mean ± SD N = 8	Candesartan mean ± SD N = 8	Control mean ± SD N = 8	Candesartan mean ± SD N = 8
Lower limit of CBF autoregulation (mmHg)	37 ± 10*	44 ± 13*	41 ± 14	40 ± 6

No significant differences with multiple comparison (ANOVA) test or t-test within each sodium group.

Table 3. Biochemical variables.

	High sodium		Low sodium	
	Control mean ± SD	Candesartan mean ± SD	Control mean ± SD	Candesartan mean ± SD
	N = 6	N = 8	N = 6	N = 6
K^+ (mmol/L)	4.3 ± 0.47	3.9 ± 0.59	4.1 ± 0.19	4.1 ± 0.27
Na^+ (mmol/L) *	148 ± 2.73	148 ± 0.92	144 ± 1.03	143 ± 0.98
Creatinine (mmol/L) §	13.6 ± 2.81	10.9 ± 0.75	16.5 ± 3.33	16.3 ± 4.43
Urea (mmol/L) *	5.2 ± 0.65	5.0 ± 0.47	7.5 ± 2.03	8.5 ± 2.73
	N = 8	N = 8	N = 8	N = 8
Hgb (mmol/L) *	7.6 ± 0.38	7.4 ± 0.60	8.6 ± 0.32	8.1 ± 0.58
PRC (mIU/L) *	11.1 ± 3.40	8.9 ± 2.45	70.1 ± 20.03	77.3 ± 36.94

Hgb: hemoglobin; PRC: plasma renin concentration.
Statistics:
(1) High sodium vs. low sodium chow, with controls and candesartan pooled together: *$p < 0.0001$, §$p < 0.005$ in t-test.
(2) High sodium: candesartan vs. control. No statistically significant differences.
(3) Low sodium: candesartan vs. control: for Hgb $p < 0.05$.

it was anticipated that candesartan would cause a shift in the lower limit of CBF autoregulation to the left towards lower blood pressure levels. This was observed in our recent study where we administrated candesartan to rats on normal diet.[5] Further, our group has previously shown that the effect of the ACEi captopril on autoregulation of CBF is independent of circulating renin. This was shown in rats that were kept alive with peritoneal dialysis for 48 h after bilateral nephrectomy. At that point circulating renin was no longer present, but the shift of the lower limit of CBF autoregulation to the left, towards lower blood pressure was preserved during ACEi with captopril, most likely due to an effect on the RAS in the vessel wall.[9] A similar effect on the lower limit of CBF autoregulation was expected in animals that had very low PRC due to high sodium intake. Vessel wall renin, on the other hand, would also expectedly be suppressed in these animals, and the lack of effect of candesartan on the lower limit of CBF autoregulation in high salt animals may simply be due to the suppression of the RAS leaving no room for pharmacological blockade.

Low sodium diet increased PRC as expected, but contrary to expectation candesartan had no effect on the lower limit in these animals either. Low sodium intake increases sympathetic activity in man and rat.[16,17] High sympathetic

Table 4. Weight and blood pressure.

	High sodium		Low sodium	
	Control mean ± SD N = 8	Candesartan mean ± SD N = 8	Control mean ± SD N = 8	Candesartan mean ± SD N = 8
Weight at start of special chow (g)	238 ± 24.06	235 ± 8.01	237 ± 10.95	246 ± 20.43
Weight at day of experiment (g)	259 ± 22.24	258 ± 9.46	249 ± 7.04	264 ± 19.27
Weight gain (%) *	8.91 ± 4.07	9.73 ± 3.36	5.25 ± 2.29	7.75 ± 2.81
MABP (mmHg) *	83 ± 12.10	84 ± 16.48	104 ± 8.00	99 ± 15.84

MABP: mean arterial blood pressure measured after anesthesia, but before craniotomy and medication with norepinephrine and candesartan.
Statistics:
(1) High sodium vs. low sodium chow, with controls and candesartan pooled together: * $p < 0.05$ in t-test.
(2) High sodium: candesartan vs. control. No statistically significant differences.
(3) Low sodium: candesartan vs. control. No statistically significant differences.
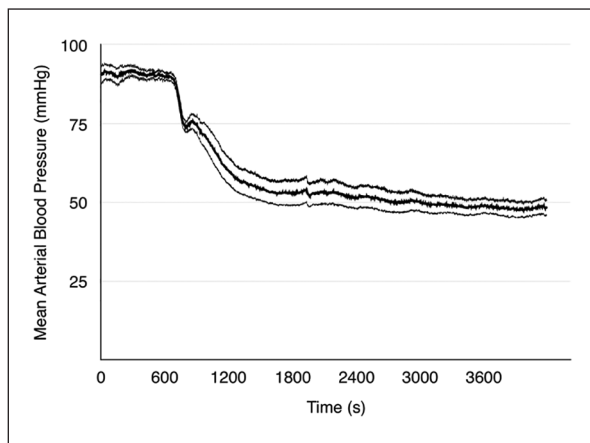
Figure 3. The isolated effect of candesartan on the arterial blood pressure. No other medication or intervention was applied. Mean arterial blood pressure dropped from about 90 mmHg to about 50 mmHg following candesartan and remained at that level for 2 h.

activity was most likely also present in the low-salt animals of the present study, given the surprising finding that blood pressure under anesthesia was on average 19 mmHg higher in low-salt than in high-salt animals despite signs of a reduced extracellular volume in the former. In these animals, a marked alpha-adrenergic sympathetic effect on the "inflow tract" arteries may have overridden the effect of the RAS and its blockade on the lower limit. Interestingly, stimulation of the sympathetic cervical ganglia has been shown to eliminate the effect of captopril on the upper limit of CBF autoregulation.[18]

In the present study, the high and very low sodium chow was given for one week before studying CBF autoregulation. In rats, increases in indices of oxidative stress may occur as a result of administration of doses of AngII mimicking activation of the renin system,[19] as well as of a high-sodium diet associated with suppression of the renin system.[20] It is possible, therefore, that elevated oxidative stress may be a common denominator underlying the present results. However, separate studies are required to elucidate this intriguing possibility.

There were several signs of expected changes in body fluid volume in the animals, in contrast to the paradoxical difference in blood pressure. In low-sodium animals, hemoglobin, creatinine and urea were significantly elevated compared to the high-sodium animals. This most likely represents intravascular depletion with reduced intravascular volume which leads to reduced kidney function and thereby elevated creatinine levels. It is well known that in rats, sodium restriction leads to marked reductions in glomerular filtration rate.[21,22] In the high-sodium animals, a significant increase in body weight was observed during the one week of diet. They also had an increased water consumption compared to the low-sodium animals, although the exact fluid intake and urinary output was not measured. Hence, the low-sodium animals expectedly would have a lowered extracellular volume and high-sodium animals an increased extracellular volume. However, it is unlikely that these changes influenced CBF and its autoregulation.

Finally, a type 2 error should be considered as a possible due to the limited number of animals in the present study. We do however find this unlikely as the results from our study do not show any trend at all towards significant difference between the two groups that were either fed high or low sodium chow or in the four subgroups that were treated with the ARB or were not treated.

Clinical implications from the present study ought to be drawn with precautions. Although it is widely accepted that salt intake should be reduced in hypertension a strict relation between salt intake and cardiovascular mortality is lacking firm evidence and a tendency to an inverse relationship has even been observed.[23] In the present study a very low salt intake was applied (0.004%) and such low salt intake are hardly seen in the clinical setting. Still our result leads to

precaution regarding massive reduction in salt intake in patients and calls for further clinical investigations.

Conclusion

Both high and low sodium diet eliminated the effect of the ARB candesartan on the lower limit of CBF autoregulation. The precise mechanisms for this are unclear, but we propose the following explanations: In the low-sodium group, blood pressure was almost 20 mmHg higher under anesthesia than in the high-sodium group, most likely because of alpha-adrenergic sympathetic activation. Such activation might override the effect of the RAS and its blockade, explaining the lack of effect of candesartan. In the high-sodium group, the RAS may have been so suppressed that it was not able to react to pharmacological blockade, explaining the lack of candesartan in this group. Clearly, further studies are necessary to confirm and explain the observations of the present work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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