Cerebrovascular Effects of Angiotensin Converting Enzyme Inhibition Involve Large Artery Dilatation in Rats

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Background and Purpose: The aim of the study was to selectively examine the effects of converting enzyme inhibition on the large brain arteries by using concomitant inhibition of carbonic anhydrase to cause severe dilatation of mainly parenchymal resistance vessels.

Methods: Cerebral blood flow was measured using the xenon-133 injection technique in three groups of Wistar rats either during carbonic anhydrase inhibition with acetazolamide (treatment A, n=8), during carbonic anhydrase inhibition followed by converting enzyme inhibition with captopril 40 minutes later (treatment B, n = 10), or during carbonic anhydrase inhibition preceded by converting enzyme inhibition 20 minutes earlier (treatment C, n=7).

Results: After treatment A, cerebral blood flow rose rapidly and stabilized within 20 minutes at an average of 220 ml/100 g • min; flow remained stable until at least 60 minutes. After treatment B, cerebral blood flow increased by a further 17.4%, from an average of 219 ml/100 g • min to an average of 257 ml/100 g • min (p<0.01). After treatment C, cerebral blood flow stabilized at an average of 238 ml/100 g • min, with flow from 20 to 60 minutes always being higher (from 5% to 17%) than during carbonic anhydrase inhibition alone (p<0.02). Thus the additional inhibition of converting enzyme resulted in higher cerebral blood flow than during inhibition of carbonic anhydrase alone.

Conclusions: These results suggest that converting enzyme inhibition reduced resistance of large brain arteries and support the hypothesis that there is some angiotensin II-induced tone in large cerebral arteries. (Stroke 1991;22:1362-1368)

A major component of peripheral large artery resistance is due to angiotensin II produced locally within the wall of large arteries rather than to circulating angiotensin II. The vascular renin-angiotensin system has also been suggested to play a role in the resistance of large cerebral arteries. Cerebral vessels have the components of a renin-angiotensin system and large pial arteries in vitro and the cerebral circulation as a whole in vivo are able to convert angiotensin I to II. Furthermore, large brain arteries are able to constrict when exposed to angiotensin I, and this constriction is severely impaired by angiotensin converting enzyme (ACE) inhibition. In contrast, circulating angiotensin II does not affect cerebral blood flow (CBF) even by intracarotid infusion, probably because of the blood–brain barrier. Although a recent study indicates that circulating angiotensin II may affect cerebrovascular resistance, the study did not distinguish between intracranial and extracranial resistance, and it is thus unclear whether intracranial or extracranial arteries are involved.

Acute inhibition of ACE has a clear-cut effect on CBF autoregulation. Although resting CBF is not influenced, the lower limit of autoregulation is reset to lower blood pressure levels in rats and humans. The effect of ACE inhibition on CBF autoregulation was interpreted as being due to release of cerebrovascular tone caused by angiotensin II produced locally in the wall of large cerebral arteries: resultant compensatory autoregulatory constriction of smaller downstream arterioles would keep CBF unchanged, but the arterioles would then have an enhanced capacity to dilate during a blood pressure fall but a reduced capacity to constrict during a pressure increase.
The object of the present study was to try to separate the effect of ACE inhibition on large cerebral arteries from the resultant compensatory downstream constriction. We reasoned that if we maximally dilated the parenchymal resistance vessels using carbonic anhydrase inhibition with acetazolamide, any subsequent release of angiotensin II-induced tone in large cerebral arteries might lead to a further increase in CBF. As we have recently observed an approximately 8% increase in pial artery pressure following administration of ACE inhibitors in rat, and as CBF in the rat is pressure passive during maximal dilatation, the expected CBF increase after ACE inhibition should also be approximately 8%. As this is the same as the variability of our method of measuring CBF, we approached the problem in two ways.

Materials and Methods

The experiments were performed on 3-month-old normotensive male Wistar rats (weight, 250–320 g) supplied by Møllegaard Ltd., Denmark. Cerebral blood flow was measured using the intra-arterial xenon-133 injection technique modified for rat studies. All data are given as the mean±SD. Statistical analysis was performed with the SPSS software package.

In group A (n = 8) we approached the problem in two ways. In the first dilated the parenchymal vessels by carbonic anhydrase inhibition and then investigated whether CBF increased following ACE inhibition. In the second part of the study we first inhibited ACE and then examined whether subsequent carbonic anhydrase inhibition caused a greater increase in CBF than with carbonic anhydrase inhibition alone.

In group B (n = 10) had carbonic anhydrase inhibition followed by ACE inhibition. Acetazolamide (30 mg/kg i.v.) was administered, and CBF was measured after 2, 10, 20, 30, 40, 50, and 60 minutes. Group B (n = 10) had carbonic anhydrase inhibition followed by ACE inhibition. Acetazolamide (30 mg/kg i.v.) was administered, and CBF was measured after 2, 10, 20, 30, 40, and 40 minutes. Captopril (10 mg/kg i.v.) was then administered, and mean arterial pressure was prevented from falling by infusion of norepinephrine (3–24 μg/kg/min). Cerebral blood flow was measured again after 5 and 10 minutes (45 and 50 minutes after acetazolamide). Mean arterial pressure was then actively manipulated by means of norepinephrine infusion or hemorrhage, and three or four CBF measurements were obtained at various mean arterial pressure levels between 70 and 130 mm Hg, with CBF being measured at about 5-minute intervals (the short interval was possible because of the high flow). As the dose of norepinephrine needed to maintain mean arterial pressure after both carbonic anhydrase and ACE inhibition was relatively high (up to 24 μg/kg/min), we checked that the infusion of norepinephrine per se did not influence CBF: in an additional four rats, 40 minutes after carbonic anhydrase inhibition, 24 μg/kg/min norepinephrine was infused, and at the same time, blood was withdrawn so as to maintain a constant mean arterial pressure. Cerebral blood flow was unchanged by this manipulation (222 ml/100 g · min before and 220 ml/100 g · min after).

In group C (n = 7), ACE inhibition was followed by carbonic anhydrase inhibition. Captopril (10 mg/kg i.v.) was administered, and 20 minutes later, when mean arterial pressure had returned to baseline levels, acetazolamide (30 mg/kg i.v.) was administered. Cerebral blood flow was measured after 20, 30, 40, 50, and 60 minutes.

All data are given as the mean±SD. Statistical analysis of CBF and mean arterial pressure changes within each group was made by one-way analysis of variance together with the Dunnet multiple comparison test. Intergroup statistical comparisons were made using Student's t test for unpaired data. Differences were accepted as significant at p < 0.05.
TABLE 1. Cerebral Blood Flow and Mean Arterial Pressure in Wistar Rats During Carbonic Anhydrase Inhibition With Acetazolamide (30 mg/kg) (Group A) and With Angiotensin Converting Enzyme Inhibition Either Superimposed on (Group B) or Preceding (Group C) Carbonic Anhydrase Inhibition

<table>
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<tr>
<th>Time (min)</th>
<th>Group A (n=8)</th>
<th>Group B (n=10)</th>
<th>Group C (n=7)</th>
<th>CBF</th>
<th>MAP</th>
<th>CBF</th>
<th>MAP</th>
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Data are mean±1 SD. In group B, captopril (10 mg/kg) was injected at 40.5 minutes. CBF, cerebral blood flow (ml/100 g · min); MAP, mean arterial pressure (mm Hg).

*p<0.02 for pooled 20–60-minute values as compared with group A.

*p<0.01 when compared with 20–60-minute values by analysis of variance and the Dunnet multiple comparison test.

Results

Cerebral blood flow and mean arterial pressure measurements in the three groups of rats are given in Table 1. In each group, carbonic anhydrase inhibition resulted in a rapid increase in CBF, with the maximum flow being reached within 20 minutes.

In group A, CBF rose from 69 ml/100 g · min and stabilized at an average of 219 ml/100 g · min from 20–60 minutes. Mean arterial pressure fell slightly within 2–10 minutes but spontaneously returned to baseline by 20 minutes. All measurements used for the comparison of CBF during carbonic anhydrase inhibition with and without ACE inhibition were thus made between 20 and 60 minutes from administration of acetazolamide, that is, during stable mean arterial pressure and CBF.

In group B, in which captopril was injected 40 minutes after carbonic anhydrase inhibition and at a time when CBF had already stabilized at a level averaging 219 ml/100 g · min, flow increased further to an average of 257 ml/100 g · min after 5 and 10 minutes, an increase of 17.4%. This increase was statistically significant (p<0.01) when compared by one-way analysis of variance to the CBF plateau from 20–40 minutes after carbonic anhydrase inhibition. In contrast, flow remained constant in the rats in group A. The results for groups A and B are given in Table 1 and illustrated by Figure 1.

In group C, in which ACE inhibition preceded carbonic anhydrase inhibition by 20 minutes, CBF stabilized at an average of 238 ml/100 g · min, with CBF from 20 to 60 minutes always higher (from 5% to 17%) than CBF at the same times in group A.

The pooled data for CBF measured between 20 and 60 minutes were significantly higher in group C than in group A (p<0.02). Simple linear regression of CBF versus time for all CBF measurements made between 20 and 60 minutes in groups A and C gave regression lines almost parallel to the time axis with constants of 220 ml/100 g · min for group A and 239 ml/100 g · min for group C. Cerebral blood flow was thus stable from 20–60 minutes after carbonic anhydrase inhibition.

In all three groups, Paco2 increased from the resting level of approximately 40 mm Hg to a plateau of approximately 60 mm Hg in the sampled blood, with pHa falling from approximately 7.42 to 7.24. However, after carbonic anhydrase inhibition it is not possible to determine accurately the circulating arterial Pco2 and pH from sampled blood because carbonic acid dehydration is retarded so much that equilibrium between bicarbonate and CO2 is not reached during the passage of blood from the lung to the brain; however, equilibrium is reached ex vivo,
was independent of mean arterial pressure (70–130 mm Hg) during carbonic anhydrase inhibition alone (group A, ○; linear regression: n=66, r=0.014, NS; CBF=174.5+0.442MAP [—]) and during inhibition of both carbonic anhydrase and angiotensin converting enzyme (groups B and C; ●; linear regression: n=71, r=0.263, p<0.025; CBF=14+2.430MAP [—]). Only data obtained at least 20 minutes after acetazolamide injection were included.

and consequently, the determined values overestimate in vivo PCO₂ and underestimate in vivo pH. Because the rats were mechanically ventilated in the same way, there are unlikely to be any major differences between the true in vivo values in the groups.

Thus, when ACE inhibition was superimposed on carbonic anhydrase inhibition, under conditions of stable mean arterial pressure and blood gases, CBF was always higher than during carbonic anhydrase inhibition alone. Since the difference in CBF between the two situations could be expected to be even greater at high blood pressure, we compared the relationship between CBF and mean arterial pressure during carbonic anhydrase inhibition alone with that during both ACE and carbonic anhydrase inhibition. This is illustrated by Figure 2, which is based on the pooled data obtained at least 20 minutes after acetazolamide administration, that is, when the effects of carbonic anhydrase inhibition were maximal and stable. The ACE plus carbonic anhydrase data include measurements from group C and measurements obtained in group B during deliberate raising and lowering of blood pressure. Simple linear regression analysis showed that CBF was independent of mean arterial pressure (70–130 mm Hg) during carbonic anhydrase inhibition alone, the slope of the regression line being essentially zero (r=0.014, NS). In contrast, during combined ACE and carbonic anhydrase inhibition CBF increased progressively with mean arterial pressure, the slope of the regression line deviating significantly from zero (r=0.263, p<0.025).

Discussion

The main finding of the present study is that higher CBF levels are reached when ACE inhibition is either superimposed on, or precedes, carbonic anhydrase inhibition with acetazolamide. Carbonic anhydrase inhibition leads to selective dilatation of mainly parenchymal resistance vessels. In awake humans and in anesthetized animals, acetazolamide has been shown to lower extracellular pH in the brain and to markedly increase CBF through the extravascular pH change.22-33-35 The present finding that acetazolamide caused a long-lasting increase in CBF of about 300% is in agreement with previous animal studies of the effect of carbonic anhydrase inhibition on CBF.24,36-39 This large increase partly reflects the fact that the animals are mechanically ventilated at a constant rate; resultant CO₂ retention and concomitant pH reduction (because spontaneous hyperventilation is not possible) probably accounts for the high flow. Such a high increase is not seen in awake humans, where CBF only doubles following maximal carbonic anhydrase inhibition.20,21,23,25

The finding that superimposition of ACE inhibition on carbonic anhydrase inhibition leads to a further 17% CBF increase (Figure 1) can be explained if carbonic anhydrase inhibition, by changing perivascular pH in brain parenchyma, results in maximal dilatation of parenchymal resistance vessels; any subsequent reduction in upstream resistance would result in a further CBF increase. In a similar way, the greater CBF increase seen when ACE inhibition precedes carbonic anhydrase inhibition can be explained by an increase in downstream pressure resulting from ACE-induced dilatation of large brain arteries. Because of the higher intravascular pressure, CBF would be greater than with carbonic anhydrase inhibition alone. The CBF plateau after acetazolamide was about 10% higher in the rats pretreated with ACE inhibition (Table 1), this being in line with the CBF increase that could be predicted from our recent observation that pial artery pressure in hypertensive rats increases approximately 8% following ACE inhibition.26

The finding that CBF was independent of mean arterial pressure in the range 70–130 mm Hg during carbonic anhydrase inhibition alone (Figure 2) may indicate that the larger upstream arteries are able to autoregulate CBF when parenchymal resistance vessels are severely dilated. This finding is in contrast to the situation with direct vasodilators such as dihydralazine, which also dilate the large brain arteries40 and with which CBF becomes pressure passive at high doses.27 That ACE inhibition removed some of the upstream large artery resistance is further evidenced by the finding that CBF increased progressively with mean arterial pressure in the range 70–130 mm Hg during combined ACE and carbonic anhydrase inhibition (Figure 2); that is, the autoregulatory capacity of the large cerebral arteries was lost.

Angiotensin converting enzyme plays an important role in two different vasoactive peptide systems. Apart from converting angiotensin I to II, ACE (or kinase II) also plays an important role in the kal-
likrein-kinin system by inactivating the hypotensive vasodilator bradykinin. Thus, the possibility that the effect of ACE inhibition might be caused by augmentation of bradykinin-induced dilatation of large brain arteries must be considered. This does not seem to be the explanation for the reduction in large artery resistance, however. First, although large brain arteries dilate in response to the application of bradykinin both in vitro and in vivo,\textsuperscript{10,41,42} it is unclear whether bradykinin is generated in brain arteries in vivo. Second, neither the application of captopril to brain arteries in vitro\textsuperscript{10,41} nor perivascular administration of captopril in vivo\textsuperscript{41} augments the dilatation of large brain arteries by bradykinin. This is probably because bradykinin can be degraded by a number of other kinases apart from ACE (kinase II). In contrast, the same in vitro study\textsuperscript{10} showed that large brain arteries are able to constrict to angiotensin I and that the constriction was severely impaired by ACE inhibition. Thus, whereas ACE in large arteries is not important for the breakdown of the vasodilator bradykinin, it is important for the conversion of angiotensin I to the vasoconstrictor angiotensin II.

Because angiotensin also acts to amplify sympathetic signals, the possibility exists that by inhibiting angiotensin II production, sympathetic constriction of large brain arteries might be attenuated, with resultant dilatation. This is unlikely to be the explanation, however, because the present experiments were conducted under halothane anesthesia using suxamethonium as the muscle relaxant, both of which have sympathetic and ganglion-blocking properties that eliminate sympathetic effects on large brain arteries in this preparation.\textsuperscript{27} Furthermore, although marked sympathetic stimulation can overcome the ACE inhibition–induced dilatation of large brain arteries and return the upper limit of autoregulation toward normal,\textsuperscript{43} the effect of ACE inhibition on CBF autoregulation is still seen after sympathetic denervation.\textsuperscript{44} Thus, the effect of ACE inhibition is unlikely to be caused by attenuation of sympathetic constriction of large brain arteries.

We thus interpret our findings to indicate that ACE inhibition increased CBF during carbonic anhydrase inhibition by causing a reduction in the resistance of large brain arteries, and that this was due to release of angiotensin II–induced tone following inhibition of angiotensin II production. This supports our hypothesis that there is some angiotensin II–induced tone in large cerebral arteries\textsuperscript{6,14,15} and indicates that large cerebral artery resistance might be partially mediated by angiotensin II.

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References


KEY WORDS • carbonic anhydrase • cerebral arteries • cerebral blood flow • renin-angiotensin system • rats
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