Abnormal Vasoconstrictor Responses to Angiotensin II and Noradrenaline in Isolated Small Arteries From Patients With Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL)

Monira B. Hussain, PhD; Sumeet Singhal, BM, BCh, BSc; Hugh S. Markus, BMBCh, MD, FRCP; Donald R.J. Singer, BMedBiol, MD, FRCP

Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterized by ultrastructural abnormalities in small cerebral and systemic vessels. We assessed vasomotor function in systemic small arteries in CADASIL.

Methods—We studied 10 CADASIL patients and 10 control subjects. Resistance arteries isolated from gluteal biopsies were mounted on small-vessel myographs, and concentration responses were determined for vasoconstrictors (noradrenaline, angiotensin II, and endothelin-I) and vasodilators (acetylcholine, bradykinin, spermine-NONOate, and nifedipine). Maximum data are shown as percent potassium contraction.

Results—There was reduced potency for noradrenaline in CADASIL (CADASIL [38 arteries]: EC50, 240 nmol/L; control subjects [27 arteries]: EC50, 100 nmol/L; 2-way analysis of variance, F = 9.76, P = 0.002). Maximum response to angiotensin II was greater in CADASIL (120 ± 8% versus 97 ± 5% in control subjects; F = 4.28, P = 0.043). Tachyphylaxis to angiotensin II occurred in all control subjects studied but in only 3 of 9 CADASIL subjects (P = 0.011, Fisher’s exact test). Vasodilation was similar in CADASIL patients compared with control subjects for endothelium-dependent dilators (acetylcholine and bradykinin) and endothelium-independent dilators (spermine-NONOate and nifedipine).

Conclusions—These results suggest a selective systemic microvascular vasoconstrictor abnormality in CADASIL in noradrenaline and angiotensin II pathways that is not explained by vasodilator impairment in endothelium or vascular smooth muscle. This could have important implications for prophylaxis and treatment of CADASIL. (Stroke. 2004;35:853-858.)

Key Words: angiotensins ■ arteries ■ CADASIL ■ muscle, smooth ■ noradrenaline
ligands to other normal receptors, reducing normal pathway activation.\textsuperscript{8}

Human Notch3 carrying an archetypal Arg90Cys mutation has been expressed in VSMCs in transgenic mice,\textsuperscript{9} which develop 2 hallmarks of the CADASIL angiopathy, GOM deposits and Notch3 accumulation, within cerebral and peripheral arteries. They show disrupted anchorage of VSMCs to adjacent extracellular matrix and cells and early signs of VSMC degeneration, which precedes accumulation of Notch3 and GOM.\textsuperscript{9}

Notch3 is constitutively expressed in smooth muscle in peripheral arteries,\textsuperscript{4} and nocturnal dipping of blood pressure is blunted in subjects with CADASIL.\textsuperscript{10} We therefore assessed vasoconstrictor function in systemic small arteries from CADASIL patients compared with control subjects well matched for cardiovascular risk factors.

**Subjects and Methods**

Subjects were recruited from a national CADASIL clinical service and were excluded if they had had stroke-like events in the 3 previous months. Control subjects were healthy volunteers from the community or hospital matched for age, sex, and blood pressure. Exclusion criteria for control subjects included previous cerebrovascular or cardiovascular events. Twelve CADASIL patients and 12 healthy volunteers were recruited. In 2 patients and 2 control subjects, insufficient material was obtained for vascular studies. Therefore, 10 CADASIL patients and 10 control subjects were studied. This study conformed to the principles outlined in Declaration of Helsinki\textsuperscript{11} and was approved by the local Hospital Research Ethics Committee. Written informed consent was obtained.

**Diagnosis and Assessment of CADASIL**

The Expanded Disability Status Scale (EDSS) and lesion load on MRI scan were used to assess disease severity of CADASIL. The EDSS is used routinely to assess disability from multiple sclerosis, another neurological disease in which disability results primarily from damage to white matter structures. On this scale, a score of 0 means the absence of symptoms or signs of disease and 10 is death as a result of disease.\textsuperscript{12} Lesion load was determined quantitatively from digitized axial T2-weighted MRI scans with in-house software (T.R. Barrick, St George’s Hospital Medical School). Hyperintense lesions, brain circumference, and lateral ventricles for all supra-tentorial slices were identified and circumscribed with image analysis software (Dispunc, D.L. Plummer, University College London). Brain parenchymal area was calculated from the difference between brain circumferential and lateral ventricle areas; total lesion load was expressed as percentage of brain parenchyma.

**Clinical Assessment**

Smoking history, height, and weight were recorded. Sitting blood pressure and pulse were measured with an Omron automated sphygmomanometer.\textsuperscript{13} Subjects were classified as hypertensive if they had a history of hypertension, were on treatment for high blood pressure, or had a blood pressure $>140/85$ mm Hg on the study day. Random serum cholesterol, homocysteine, and blood glucose were measured with standard methods. Fasting glucose was checked. Diabetes was defined as fasting glucose $>6.0$ mmol/L or history of diabetes. Hypercholesterolemia was defined as random serum level $>5.6$ mmol/L based on the reference range of our biochemistry laboratory.

**Myograph Studies**

Subjects were asked to discontinue any antplatelet agents or cholesterol-lowering drugs for 72 hours before biopsy and blood pressure-lowering drugs 12 hours before biopsy. Smokers were asked not to smoke on the day of biopsy. Resistance arteries were dissected from subcutaneous fat obtained from gluteal biopsies of skin and adipose tissue obtained under local anesthesia (2% lignocaine). Each biopsy was collected in HEPES, stored overnight at 4°C, and studied the next day.\textsuperscript{14}

Multiple arteries were obtained from different biopsies for construction of concentration-response curves (CRCs) to the reagents noted below (Figure 1). Arterial rings were mounted on a small-vessel myograph (Danish Myotech) in Krebs’ solution at 37°C and bubbled with 95% O$_2$/5% CO$_2$. Vessels were equilibrated for 1 hour and normalized to 90% of the diameter achieved at a transmural pressure of 100 mm Hg.\textsuperscript{14} Arterial segments were repeatedly exposed to high potassium solution (K$_+$: 124 mmol/L potassium chloride solution) until reproducible responses were obtained (within 10%). Tissue was discarded if K$^+$ contractions were less than during normalization.

Half-log molar incremental CRCs to noradrenaline were obtained. After 30 minutes of washing, tissues were preconstricted to noradrenaline to give a 70% to 80% response. CRCs were then obtained with endothelium-dependent (acetylcholine or bradykinin) or endothelium-independent (spermine-NONOate) vasorelaxants. Each concentration of nifedipine in contact with tissue for 4 minutes.

**Data and Statistical Analyses**

Responses to noradrenaline, angiotensin II, and endothelin-1 are represented as percentage maximum K$^+$ contraction. Responses for vasodilators are given as percentage reversal of preconstricted noradrenaline tone. The antilogarithm of half-maximal response concentrations ($pEC_{50}$, potency of response) for vasoactive agents was calculated from nonlinear regression curve fitting with GraphPad Prism. CRCs between CADASIL patients and control subjects were compared by 2-way analysis of variance for repeated measures, with Bonferroni’s post-hoc testing when appropriate. Pearson’s correlation coefficient ($r$) was used for parametrically distributed variables. For potency and EDSS score, we used Spearman’s correlation coefficient ($p$). We compared differences in tachyphylaxis between patients and control subjects using Fisher’s exact test.

![Figure 1. Pathways studied.](http://stroke.ahajournals.org/content/38/8/1891.full)
TABLE 1. Demographic and Blood Results in CADASIL (n=10) and Controls (n=10)

<table>
<thead>
<tr>
<th></th>
<th>CADASIL Patients (n=10)</th>
<th>Control Subjects (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47±4</td>
<td>50±4</td>
<td>0.70</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>123±6/76±4</td>
<td>118±3/80±2</td>
<td>0.55/0.45</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2±5.2</td>
<td>28.1±4.8</td>
<td>0.47</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.2±0.3</td>
<td>5.6±0.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>13.7±2.2</td>
<td>12.1±1.5</td>
<td>0.57</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.0±0.2</td>
<td>5.0±0.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Current/ex-smoker, n</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypertensive, n</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Antihypertensive therapy, n</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia, n</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Statin therapy, n</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM when appropriate.
*Lisinopril 10 mg OD in both cases.
†Simvastatin 10 mg, fluvastatin 40 mg, atorvastatin 10 mg, or pravastatin 20 mg, all OD.

Results

The diagnosis of CADASIL was made from a known Notch3 gene mutation in all cases. All CADASIL subjects were symptomatic; 3 had experienced only migraine with aura. Patients and control subjects were well matched for age, sex, and cardiovascular risk factors (Table 1). Treatments for blood pressure and raised cholesterol are shown in Table 1. All patients were taking aspirin, dipyridamole, or both. One patient each was taking amitriptyline 10 mg, sertraline 50 mg, and venlafaxine 150 mg. Three were taking folic acid. No control subjects were taking vasoactive drugs.

Responses to Vasoconstrictors

Contractions to 124 mmol/L potassium chloride reached a plateau within 2 minutes, with similar maximum contraction for CADASIL subjects (7.9±0.7 mN; 38 vessels from 10 subjects) and control subjects (8.1±0.8 mN; 27 vessels from 10 subjects). Potency for noradrenaline contraction was reduced: EC50 for noradrenaline was greater in CADASIL patients (7.9±0.7 mN; 38 vessels from 10 subjects) than control subjects (8.1±0.8 mN; 27 vessels from 10 subjects). EC50 for noradrenaline was 8.6±0.1 [240 mmol/L]; control pEC50, 7.0±0.2 [100 mmol/L]; F=9.76, P=0.002; Figure 2a and Table 2). Maximum contractile response to angiotensin II was also greater in CADASIL patients (120±8%) compared with control subjects (97±5%, F=4.28; P=0.043; Figure 2b and Table 2). Tachyphylaxis to angiotensin II was present in only 3 of 9 CADASIL subjects but occurred in all 6 control subjects tested (P=0.011, Fisher’s exact test).

Responses to Vasodilators

Within treatments, for the endothelium-dependent vasodilators acetylcholine and bradykinin (Figure 3a and Table 2) and the endothelium-independent vasodilators spermine-NONOate and the L-type calcium channel antagonist nifedipine (Figure 4a and Table 2), there was similar reversal of noradrenaline precontracted tone in CADASIL patients compared with control subjects.

Relationships Between Vasoactive Responses and Cardiovascular Risk Factors

There were no age-related changes in vasoactive responses. Subjects with higher body mass index had reduced maximum relaxant response to angiotensin II (r=−0.564, P=0.023). Serum cholesterol was correlated with potencies for noradrenaline (r=0.683, P=0.002), angiotensin II (r=0.541, P=0.031), and endothelin-1 (r=0.520, P=0.027).

Relationships Within Vasoactive Responses

There was a significant direct correlation between efficacy (maximum vasoconstrictor response) to angiotensin II and to endothelin-1 (r=0.83, P=0.019). The pEC50 for relaxation to the vasodilator bradykinin was positively related to that for spermine-NONOate (r=0.94, P<0.0001). However, efficacy (maximum vasodilation) for acetylcholine was inversely related to that for nifedipine (r=−0.76, P=0.028).

Relationships Between Vasoactive Responses and Severity of CADASIL

The severity of CADASIL involvement as assessed by the EDSS was positively correlated to pEC50 for endothelin-1 (r=0.64, P=0.048) and noradrenaline (r=0.82, P=0.004). The EDSS was inversely associated with the maximum contractile response to angiotensin II (r=−0.71, P=0.032). Lesion load was positively correlated with noradrenaline.
pEC\textsubscript{50} (p=0.806, P=0.005) and endothelin-1 pEC\textsubscript{50} (p=0.648, P=0.043).

**Discussion**

This study demonstrated selective abnormalities in vasoconstrictor reactivity in systemic small arteries from subjects with CADASIL, an important inherited cause of premature stroke and dementia. We found both increased maximal responsiveness to the constrictor effects of angiotensin II and reduced sensitivity to the constrictor effects of the catecholamine noradrenaline, but we saw no difference in endothelin-1 responsiveness. Because there were no major differences in response to a wide range of endothelium-dependent and -independent vasodilators, our findings point to functional abnormalities in CADASIL within vasoconstrictor pathways in systemic small artery smooth muscle rather than indirect effects resulting from altered vasorelaxant pathways. These results support our hypothesis regarding impaired vasoreactivity of small arteries in peripheral tissue in CADASIL.

Our study is the first to provide direct evidence for abnormal vasoconstrictor reactivity in the systemic arterial circulation in CADASIL. Previous studies had reported impaired cerebral vasoreactivity in CADASIL as determined by blunted vasodilation to inhaled carbon dioxide\textsuperscript{15} or the carbonic anhydrase inhibitor acetazolamide.\textsuperscript{16} There has been possible indirect evidence for impaired vasoactivity in the systemic circulation from a study using ambulatory blood pressure monitoring\textsuperscript{10} that reported blunting of the normal nocturnal blood pressure dip in subjects with CADASIL compared with matched control subjects. However, abnormal central sympathetic outflow could have been responsible for the relatively raised nighttime blood pressure reported. Our results provide a further systemic mechanism for impaired circadian blood pressure regulation in CADASIL: abnormal vasoconstrictor responses in systemic small arteries, a major contributory site for regulation of systemic vascular resistance. The resulting increase in cumulative daily blood pressure load for a given office blood pressure could contribute to the pathogenesis of arterial disease in CADASIL.

We identified 2 major abnormalities in angiotensin II responsiveness in CADASIL. First, there was an increase in the maximum vasoconstrictor response in CADASIL patients compared with control subjects. This suggests greater bioactivity of the angiotensin II signal transduction pathway. We excluded impaired vasodilator responsiveness as a mechanism for this because relaxation to 2 different endothelium-dependent dilators, acetylcholine and bradykinin, was similar

<table>
<thead>
<tr>
<th>Drugs</th>
<th>CADASIL Patients pEC\textsubscript{50} (EC\textsubscript{50}, nmol/L)</th>
<th>Control Subjects pEC\textsubscript{50} (EC\textsubscript{50}, nmol/L)</th>
<th>CADASIL Patients Maximal</th>
<th>Control Subjects Maximal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>6.6±0.1 (240)</td>
<td>7.0±0.2 (100)</td>
<td>153±6</td>
<td>151±29</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>8.3±0.1 (5.4)</td>
<td>8.2±0.1 (6.7)</td>
<td>138±11</td>
<td>154±18</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>8.7±0.2 (2.0)</td>
<td>8.7±0.1 (2.0)</td>
<td>97±15</td>
<td>120±8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>7.8±0.1 (16.0)</td>
<td>7.9±0.1 (13.0)</td>
<td>88±3</td>
<td>96±4</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>8.3±0.1 (5.6)</td>
<td>8.2±0.1 (6.8)</td>
<td>86±4</td>
<td>94±5</td>
</tr>
<tr>
<td>Spermine-NONOate</td>
<td>6.6±0.1 (230)</td>
<td>6.5±0.1 (280)</td>
<td>94±7</td>
<td>104±10</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>8.4±0.3 (4.0)</td>
<td>8.2±0.3 (5.9)</td>
<td>75±8</td>
<td>73±7</td>
</tr>
</tbody>
</table>

**Figure 3.** CRCs to acetylcholine (a; Ach) and bradykinin (b; BK) in CADASIL and control subjects.

**Figure 4.** CRCs to spermine-NONOate (a; SPER-NO) and nifedipine (b) in CADASIL and control subjects.
in CADASIL compared with control subjects, as was relax-
ation to the endothelium-independent dilator nitric oxide
donor spermine-NONOate and the calcium channel blocker
nifedipine. Mechanisms for increased vasoconstriction to
angiotensin II may include increased activity of classic
angiotensin II type 1 receptor–mediated signal transduction
pathways such as activation of phospholipase C with gener-
ation of inositol phosphate, a rise in intracellular Ca²⁺, protein
kinase C activation, and alternative pathway activation such
as oxidant stress-mediated contraction through stimulation of
NAD(P)H oxidase and other oxidant enzymes.17

Second, we found marked blunting of tachyphylaxis to the
vasoconstrictor effects of angiotensin II in CADASIL. It is
well established that the normal vasoactive small artery
response to angiotensin II is initial contraction, followed by
desensitization or tachyphylaxis.18 In our study, tachyphyl-
axis to angiotensin II–mediated contraction occurred as
expected in all control subjects studied but in only 30% of the
CADASIL group. The cause is unclear, but loss of tachyphy-
laxis may have contributed to the enhanced contractile
response to angiotensin II found in the CADASIL arteries.
Tachyphylaxis has been reported to be blunted when endo-
thelial bioactivity is impaired. Postulated mechanisms for this
endothelium-dependent desensitization include mediation by
a cyclooxygenase cytochrome P450 product, which could act
by increasing K(Ca²⁺) channel activity.19 However, impaired
endothelial function does not appear to be a major explana-
tion for blunted tachyphylaxis in CADASIL because in the
present study endothelium-dependent relaxant responses to
both acetylcholine and bradykinin appeared similarly pre-
served in CADASIL subjects compared with matched control
subjects.

Angiotensin II downregulates Notch3 ligand (Jagged 1)
and Notch3 receptor expression in rat aortic smooth muscle
cells and inactivates endothelial cell–derived Jagged 1 by
glycosylation.4 In CADASIL, mutant Notch3 receptors are
not effectively downregulated. An intriguing possibility is
that increased angiotensin II vasoconstriction may reflect
general upregulation of angiotensin II pathways in response
to Notch3 accumulation in CADASIL. If so, this would
appear to be an early feature of the natural history of
CADASIL because angiotensin II maximal contraction was
inversely related to disease severity as assessed by EDSS.

Noradrenaline potency was 2.4-fold lower in CADASIL
compared with control subjects. This decreased sensitivity to
noradrenaline may reflect overactivity of sympathetic out-
flow in CADASIL. Altered α₁ adrenoceptor–mediated tone
can affect cerebral perfusion by restricting the ability of
affected vessels in CADASIL to autoregulate during periods of
low perfusion pressure.

There are structural abnormalities in small penetrating
arteries supplying subcortical structures in CADASIL, with
accumulation of GOM in the basement membrane of VSMCs
of cerebral arterioles.20 Our study provides a mechanism for
functionally impaired vasoreactivity in the form of increased
vasoconstriction to angiotensin II in CADASIL, compounded
by reduced tachyphylaxis. The resulting failure of appropriate
systemic vasoconstriction, accompanied by direct or indirect
impairment of regulation of the cerebral blood supply, could
contribute to recurrent brain hypoperfusion injury.

Although noradrenaline potency was reduced in CADASIL,
subjects with higher noradrenaline potency had greatest disease
severity for CADASIL on the basis of both EDSS and lesion
load assessed by brain imaging. One possible explanation is that
reduced noradrenaline potency may be a secondary, perhaps
compensatory, response to the primary pathogenetic insult in
CADASIL. If patients with more severe disease were less able to
suppress noradrenaline potency, a spurious relationship between
potency and disease severity may have arisen.

Further studies are needed to explore mechanisms for the
contrast between reactivity to endothelin and other vasocon-
strictors. However, there are clear precedents for dissociation
between response to endothelin and to other vasoconstrictors
in pathophysiology.21

Aspirin was stopped for only 3 days in view of the stroke
risk associated with CADASIL. Prolonged posttreatment
actions of aspirin result largely from residual effects on
platelets. In contrast, vascular cells recover rapidly from the
effects of previous aspirin treatment because unlike platelets
they can synthesize new COX enzyme.22 Confounding by
recent aspirin treatment is therefore less a concern for these
ex vivo studies than would be the case for in vivo studies.
Statins may improve endothelium-dependent relaxation23;
however, we found no evidence that altered endothelium-
dependent relaxation accounted for differences in vasocon-
strictor responses between CADASIL and control arteries.
Because only 2 of our CADASIL patients were on prior
angiotensin-converting enzyme inhibitor treatment and this
treatment was stopped at least 12 hours before biopsy, we
think it unlikely that this treatment would have greatly
influenced our findings.

Our study suggests that vasoactive consequences of the
initial pathogenetic defect in CADASIL are highly selective,
with preservation of a wide range of relaxant pathways and
selective changes in α-adrenergic and angiotensin II path-
ways. These are ex vivo studies; further in vivo studies are
needed to establish the clinical relevance of our findings. The
above observations have 2 major therapeutic implications.
They provide a focus for exploring signal transduction for
selected vasoconstrictor targets in the future treatment of
CADASIL. Second, preservation of relaxant pathways indi-
cates that there may be a wide range of endothelium-
dependent and independent dilator agents that could be used
in trials of treatment of CADASIL.

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