Changes in Vasoconstrictor and Vasodilator Responses of the Basilar Artery During Maturation in the Watanabe Heritable Hyperlipidemic Rabbit Differ From Those in the New Zealand White Rabbit

Anne L. Stewart-Lee and Geoffrey Burnstock

This investigation involved alterations in the local control of vascular tone in the isolated rabbit basilar artery in atherosclerosis, with Watanabe heritable hyperlipidemic (WHHL) rabbits as a model and New Zealand White (NZW) rabbits as controls. Vasoconstrictor responses to KCl in isolated preparations of the basilar artery at basal tone showed no differences at 4, 6, and 12 months of age in either WHHL or NZW rabbits. Contractile responses to both histamine and neuropeptide Y were significantly greater in 12-month-old WHHL rabbit preparations when compared with responses measured at 4 and 6 months. In NZW rabbit preparations, there was no change in maximum contractile responses to both histamine and neuropeptide Y over the same age range. Endothelium-dependent relaxations to acetylcholine in raised-tone preparations from WHHL rabbits were significantly greater at 6 months in comparison with responses measured at both 4 and 12 months of age. In contrast, endothelium-independent relaxations to calcitonin gene–related peptide and vasoactive intestinal polypeptide showed no change over the age range studied. In NZW rabbit preparations, both endothelium-dependent and endothelium-independent relaxations declined significantly between 4 and 12 months. The significance of these changes in the rabbit basilar artery in atherosclerosis is discussed in relation to the “protection” of intracranial arteries from atherosclerosis and their subsequent susceptibility to cerebral ischemia and stroke. (Arteriosclerosis and Thrombosis 1991;11:1147–1155)

It is generally acknowledged that the distribution of atherosclerotic lesions in blood vessels is heterogeneous. In particular, the involvement of intracranial arteries in atherosclerosis is unusual because these arteries appear to be subject to less atherosclerosis than others. Indeed, it has been reported that cerebral arteries are the last to be affected in humans as well as in various animal models subjected to diet-induced atherosclerosis, for example, rhesus and cynomolgus monkeys and rabbits, including the Watanabe heritable hyperlipidemic (WHHL) rabbit.

One of the clinical manifestations of atherosclerosis is cerebral infarction (stroke). However, many investigators have pointed out the importance of atherosclerosis in the extracranial carotid arteries as opposed to the intracranial arteries in the pathogenesis of cerebrovascular disease, particularly in light of the apparent “protection” of intracranial arteries from atherosclerosis.

The protection of the intracranial vasculature from atherosclerosis is not limited to humans and nonhuman primates. For example, in rats subjected to a short-term atherogenic diet, lesions in the cerebral vessels (circle of Willis) were virtually absent, unlike in the carotid arteries and aorta, where intimal lesions were identified. Moreover, the absence of lesions has been reported in basilar arteries of 20–34-month-old WHHL rabbits despite the presence of lesions in the aorta, in coronary arteries, and at the carotid bifurcation in the neck. Transmission electron microscopy studies in our laboratories have also shown that in the basilar artery of WHHL rabbits as old as 12 months, the
ultrastructural morphology of the majority of endothelial and smooth muscle cells appeared normal, with no evidence of lesion development.15

It is well established that the endothelium is of great importance in the regulation of vascular tone.16-18 Furthermore, it is recognized that vasodilation mediated via the release of endothelium-derived relaxing factor (EDRF) is reduced in grossly atherosclerotic vessels.19-23

In this study vasodilatory responses to acetylcholine (ACh), calcitonin gene–related peptide (CGRP), and vasoactive intestinal polypeptide (VIP) were examined in the basilar artery of WHHL rabbits and compared with those in control NZW rabbits. Vasodilator responses to ACh are known to be endothelium-dependent, whereas CGRP and VIP are both known to have potent vasodilatory actions by acting directly on the muscle in cerebral arteries, that is, in an endothelium-independent manner.24 Comparison between differences in trends in either strain of rabbit between 4 and 12 months of age enabled us to distinguish developmental changes from atherosclerosis-related changes. This particular age range was chosen because atherogenesis has been reported to occur from 5 months of age in the aorta and coronary arteries of the WHHL rabbit.25,26 This age range therefore includes the time when lesions normally appear and allows any functional changes, which may be linked to the protection of the vessel from plaque formation, to be observed.

**Methods**

**Preparation of Material**

Four-, 6-, and 12-month-old female WHHL rabbits were used in this study, with NZW rabbits acting as sex- and age-matched controls. Animals were killed by lethal injection of sodium pentobarbitone (60 mg/ml) into the marginal ear vein, followed by exsanguination. The brain was quickly exposed and removed, and the basilar artery was isolated, taking care not to cause damage to the endothelium. Two rings 4 mm long were cut from the central region of the artery and were mounted horizontally under isometric conditions in 10-ml organ baths according to the method of Bevan and Osher.27 The tissues were bathed in Krebs’ solution of the following composition (mM): NaCl 133, KCl 4.7, NaH2PO4 1.35, NaHCO3 16.3, MgSO4 0.61, glucose 7.8, and CaCl2 2.52. The Krebs’ solution was bubbled with 95% O2 and 5% CO2 and maintained at a constant temperature of 37°C. Bovine serum albumin (50 mg/l) and bacitracin (30 mg/l) were added to the Krebs’ solution to prevent peptide adherence to the surfaces of the glassware and peptide degradation, respectively. Preparations were left to equilibrate for at least 1 hour under a resting tension of approximately 0.5 g. Contractions of the circular smooth muscle were recorded by use of a Grass Model FT03C transducer and displayed on an ink-writing oscillograph (Grass model 79).

**Experimental Procedure**

KCl (120 mM) was added to the organ bath as a single dose and washed out, once a maximum response had been established. Histamine was added cumulatively to the bath (0.1-300 μM), so that a concentration–response curve could be constructed. Neuropeptide Y (NPY) (0.1 μM) was added as a single dose to vessel preparations at resting tone and washed from the bath, once the maximum response had been established.

To study relaxation, vascular tone was raised by addition of a concentration of histamine that produced approximately 60–70% of the histamine contraction obtained at 300 μM in each vessel (10 μM histamine). ACh (0.1–300 μM) did not cause desensitization in raised-tone preparations and was therefore added cumulatively to the bath. The relaxant responses of the peptides CGRP (0.01 μM) and VIP (0.3 μM) were also measured on raised-tone preparations of the basilar artery. Each peptide was added separately as a single dose (to avoid tachyphylaxis) and washed from the bath after the point of maximum response had been recorded.

At least 20 minutes was allowed between washing one drug from the bath and testing with another drug. During this time the Krebs’ solution was changed approximately every 5 minutes. Drugs were tested in the same sequence in every experiment, this being KCl, histamine, ACh, CGRP, VIP, and NPY.

**Drugs**

Histamine dihydrochloride, ACh chloride, bacitracin, and bovine serum albumin were all obtained from Sigma Chemical Co., Poole, UK. KCl was obtained from BDH Ltd., Poole, UK. NPY and CGRP were obtained from Cambridge Research Biochemicals, Cambridge, UK. Sodium pentobarbitone was obtained from RMB Animal Health Ltd., Dagenham, UK. All drugs were dissolved in distilled water, and NPY, CGRP, and VIP were stored at −20°C in aliquots containing approximately 25 μl of the peptides dissolved in sterile distilled water.

**Expression of Results and Statistical Analysis**

Contractions to KCl are expressed in grams, whereas those to histamine and NPY are expressed as percentages of the KCl-induced contractions. This procedure allowed differentiation between changes in smooth muscle contractility (KCl) and receptor-mediated changes (histamine and NPY).

Relaxations to ACh, CGRP, and VIP are expressed as percentages of relaxation of the raised-tone preparations. The pD2 values for histamine and ACh were estimated from the mean of the negative logarithmic concentration of the drug (±SEM), which produced 50% of its maximal response in each concentration–response curve. Maximal responses and pD2 values were calculated for each preparation. For each result one average value per animal was taken. In the figures results are given as mean±SEM,
Results

Vasoconstrictor Responses

KCl (120 mM) produced a rapid contraction in all vessel segments tested (Figure 1a). Within each strain of rabbit there was no significant change in the magnitude of the contraction over the age range studied (Figure 1b and Table 1).

The cumulative application of histamine (0.1–300 μM) to isolated segments of the basilar artery produced concentration-dependent contractions in all vessel segments tested. There was little variation in either the maximum contractions to histamine or the pD2 values calculated from the concentration–response curves for NZW rabbits at 4, 6, or 12 months of age (Figure 2a and Tables 1 and 2). In WHHL rabbits, however, maximum contractions to histamine increased at 12 months of age, so that they were significantly greater than the contractions measured at 4 and 6 months of age (Figure 2b and Table 1). pD2 values to histamine were not significantly different at 4, 6, and 12 months of age in WHHL rabbits (Table 2).

NPY (0.1 μM) produced a relatively small contraction in NZW rabbit preparations of the isolated basilar artery, showing no variation at 4, 6, and 12 months of age (Figure 3 and Table 1). In WHHL rabbits contractions to NPY were significantly increased at 12 months of age when compared with responses measured at 4 and 6 months of age in this strain (Figure 3 and Table 1).

Vasodilator Responses

Endothelium-dependent vasodilatation. The cumulative application of ACh (0.1–300 μM) to isolated raised-tone segments of the basilar artery produced concentration-dependent relaxations in all vessel segments tested. In NZW rabbit preparations maximum relaxations to ACh were significantly reduced at 12 months of age when compared with responses measured at both 4 and 6 months of age (Figure 4a and Table 3). There was, however, no change in the pD2 values calculated in NZW preparations at 4, 6, or 12 months of age (Table 2). In WHHL rabbit preparations, however, maximum relaxations to ACh were significantly increased at 6 months of age when compared with responses measured at 4 months of age. This increase in maximum relaxations to ACh then decreased significantly at 12 months of age in WHHL rabbits, so that it was no longer different from relaxations measured at 4 months of age (Figure 4b and Table 3). There was no variation in the pD2 values calculated from the concentration–re-
Figure 2. Isolated transverse ring preparations of the rabbit basilar artery at resting tone. Shown are cumulative concentration–response curves to histamine (0.1–300 μM) in ring preparations taken from New Zealand White (NZW) rabbits (n=9,9,9) (panel a) and Watanabe heritable hyperlipidemic (WHHL) rabbits (n=8,6,7) (panel b) at 4 (●), 6 (■), and 12 (▲) months of age, respectively. Data points are means with SEM shown by vertical bars. Significant differences (*, p<0.05; **, ++ p<0.01; *** p<0.001) between different age groups of each separate strain of rabbit were calculated by Tukey’s test after analysis of variance. *Significant differences between 4 and 12 months; †, significant differences between 6 and 12 months.

Response curves at 4 and 6 months of age in WHHL rabbit preparations, although at 12 months of age, the pD_2 value showed a significant shift to the right when compared with the average pD_2 value calculated at both 4 and 6 months of age (Table 2). Endothelium-independent vasodilatation. CGRP (0.01 μM) produced a relaxant response in isolated raised-tone preparations of the rabbit basilar artery (Figure 5a). In NZW preparations the relaxant response to CGRP declined with increasing age and was significantly reduced between 4 and 12 months of age (Figure 5b and Table 3). In WHHL rabbits, however, relaxant responses to CGRP showed no significant change between 4 and 12 months of age, although there was a slight increase at 6 months of age when compared with responses at 4 months.

VIP (0.3 μM) also produced a relaxant response in isolated raised-tone preparations of the rabbit basilar artery (Figure 6a). In NZW rabbit preparations the relaxant response to VIP was significantly reduced at 6 and 12 months of age when compared with responses measured at 4 months of age (Figure 6b and Table 3). In WHHL rabbit preparations responses increased between 4 and 6 months of age and decreased at 12 months of age, although not significantly.

Discussion
This study clearly identified various differences in the local control of vascular tone in the isolated basilar artery from atherosclerotic (WHHL) rabbits when compared with responses in the same artery from NZW rabbits, despite the lack of lesion development.4,15 Contractile responses to KCl, histamine, and NPY showed no variation over the age range studied in NZW rabbits. The absence of any change in contractile response to KCl in basilar arteries of NZW rabbits between 4 and 12 months of age suggests that full contractility of the smooth muscle has developed
TABLE 2. Statistical Analysis of pD2 Values to Acetylcholine and Histamine

<table>
<thead>
<tr>
<th>Group/age (mo)</th>
<th>Hist</th>
<th>ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZW 4 (n=9)</td>
<td>5.3±0.08</td>
<td>6.27±0.23</td>
</tr>
<tr>
<td>6 (n=9)</td>
<td>5.26±0.1</td>
<td>6.14±0.17</td>
</tr>
<tr>
<td>12 (n=9)</td>
<td>5.52±0.12</td>
<td>5.9±0.2</td>
</tr>
<tr>
<td>WHHL 4 (n=8)</td>
<td>5.53±0.07</td>
<td>6.0±0.12*</td>
</tr>
<tr>
<td>6 (n=6)</td>
<td>5.55±0.14</td>
<td>6.18±0.16†</td>
</tr>
<tr>
<td>12 (n=9)</td>
<td>5.46±0.11</td>
<td>5.32±0.2*†</td>
</tr>
</tbody>
</table>

All values are mean±SEM with the number of observations (n) in parentheses. Values were calculated from the respective concentration-response curves in isolated preparations of the basilar artery from New Zealand White (NZW) and Watanabe heritable hyperlipidemic (WHHL) rabbits at 4, 6, and 12 months of age. pD2 values were calculated from the mean negative logarithmic concentration of histamine (Hist) or acetylcholine (ACh) ±SEM, which produced 50% of the maximal response.

Significant differences (p<0.05) were calculated by use of Tukey's method within individual rabbit strains, when analysis of variance indicated significant variation within the group. Values with the same symbol are significantly different (no symbol indicates no significant difference).

by 4 months of age in these rabbits. This is consistent with a previous study of the NZW rabbit basilar artery, in which it was reported that potassium-induced contractions primarily increased with age up to 90 days old29 (our study began with ≈120-day-old rabbits).

In contrast to NZW preparations, contractile responses to both histamine and NPY increased in 12-month-old WHHL rabbit preparations, despite no change in the contractile response to KCl over the age range studied. This suggests a general upgrading of postjunctional receptors for specific constrictor agents in the 12-month-old WHHL rabbit basilar artery.

The contractile response elicited by NPY was generally small, particularly in comparison with the responses to KCl and histamine in the NZW rabbit basilar artery at all ages studied. This weak contractile response to NPY has previously been recorded in various rabbit arteries and veins, including the basilar, middle cerebral, and internal carotid arteries.30,31 It is also known that NPY coexists with norepinephrine (NE) in perivascular nerve fibers32-34 and is coreleased with NE on sympathetic nerve stimulation.35,36 In addition, NPY potentiates the contractile response of isolated blood vessels to NE, histamine, and sympathetic nerve stimulation.30,32,37,38 The small vasoconstrictor action of NPY would suggest that the potentiating effects of NPY may be the more important contribution to vasoconstriction, rather than its direct effects. However, in this study we have been able to establish the importance of the direct vasoconstrictor action of NPY in the WHHL rabbit basilar artery, as its effect increases significantly in 12-month-old rabbits.

The present study shows that in NZW rabbits, vasodilatory responses to ACh, CGRP, and VIP followed similar trends in that responses diminished with age. The decrease in ACh-mediated relaxations in NZW rabbits may be partly due to a specific decline in endothelium-mediated responses between 6 and 12 months of age because responses to VIP and CGRP, which act directly on the muscle, did not decline significantly between these two age groups. There is no general agreement as to whether endothelium-dependent responses decline with age because there seems to be much variation between species, blood vessels within species, and even the

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Isolated transverse ring preparations of the rabbit basilar artery at resting tone. Panel a: Trace showing response to neuropeptide Y (NPY, 0.1 μM) on a resting-tone preparation from a 4-month-old New Zealand White (NZW) rabbit. Panel b: Plot of responses to NPY (0.1 μM) in NZW (•, n=9,9,9) and in Watanabe heritable hyperlipidemic (WHHL, ■) rabbit preparations (n=8,6,7) at 4, 6, and 12 months of age, respectively. Data points are means with SEM shown by vertical bars.
dilator substance used. However, in the NZW basilar artery endothelium-mediated relaxations to ACh appear to decrease with increasing age.

In this study endothelium-mediated responses are specifically potentiated in the 6-month-old WHHL rabbit basilar artery because relaxations to ACh were significantly increased in 6-month-old rabbits when compared with WHHL rabbits at 4 months of age. In contrast, endothelium-independent relaxations to CGRP and VIP, although slightly increased in 6-month-old WHHL rabbits, were not significantly different when compared with responses measured in 4-month-old WHHL rabbits. In grossly atherosclerotic vessels, endothelium-mediated responses are specifically inhibited, for example, in the human coronary artery, in the fat-fed hypercholesterolemic rabbit aorta, and in the WHHL rabbit thoracic aorta. The WHHL rabbit basilar artery, however, does not follow this trend, and this may be linked to the lack of evidence for extensive lesions in this artery. In WHHL rabbits as old as 12 months, the central ear, mesenteric, hepatic, and saphenous arteries have also been found to exhibit minimal structural changes, these being confined to early signs of lesion development (such as migration of smooth muscle cells to the intima) only at branch orifice areas. These arteries like the basilar artery also showed augmented endothelium-mediated vasodilatation.

It is suggested that possible reasons for this augmentation may include increased levels of superoxide anion scavengers such as superoxide dismutase, which would result in the potentiation of EDRF action. A second possibility is that the compensatory increase in sensitivity to EDRF is the result of elevated levels of circulating low density lipoprotein (LDL) for a prolonged period of time, as LDL is...
Statistical Analysis of Relaxant Responses in Isolated Preparations of Basilar Artery Preconstricted With 10 μM Histamine From New Zealand White and Watanabe Heritable Hyperlipidemic Rabbits

<table>
<thead>
<tr>
<th>Group/age (mo)</th>
<th>NZW (n=9)</th>
<th>WHHL (n=8)</th>
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<tbody>
<tr>
<td>4</td>
<td>53.76±2.96*</td>
<td>63.28±3.38*</td>
</tr>
<tr>
<td>6</td>
<td>51.96±3.19†</td>
<td>40.64±7.89</td>
</tr>
<tr>
<td>12</td>
<td>17.54±5.59‡</td>
<td>62.05±8.99</td>
</tr>
</tbody>
</table>

All values are mean±SEM with the number of observations (n) in parentheses. Measurements were taken at 4, 6, and 12 months of age. Maximum relaxant response to acetylcholine (ACh, 0.1–300 μM), calcitonin gene–related peptide (CGRP, 0.01 μM), and vasoactive intestinal polypeptide (VIP, 0.3 μM) are shown.

NZW, New Zealand White rabbit; WHHL, Watanabe heritable hyperlipidemic rabbit.

Significance differences (p<0.05) were calculated by use of Tukey’s method within individual rabbit strains, when analysis of variance indicated significant variation within the group. Values with the same symbols are significantly different (no symbol indicates no significant difference).

Figure 5. Isolated transverse ring preparation of the rabbit basilar artery with tone raised by histamine (10 μM). Panel a: Trace showing response to calcitonin gene–related peptide (CGRP) (0.01 μM) on a raised-tone preparation from a 4-month-old New Zealand White (NZW) rabbit. Panel b: Plot of responses to CGRP (0.01 μM) in NZW (●, n=9,9,9) and Watanabe heritable hyperlipidemic (WHHL, ■) rabbit preparations (n=8,6,9) at 4, 6, and 12 months of age, respectively. Data points are means with SEM shown by vertical bars.

Figure 6. Isolated transverse ring preparation of the rabbit basilar artery with tone raised by histamine (10 μM). Panel a: Trace showing response to vasoactive intestinal polypeptide (VIP) (0.3 μM) on a raised-tone preparation from a 4-month-old New Zealand White (NZW) rabbit. Panel b: Plot of responses to VIP (0.3 μM) in NZW (●, n=9,9,9) and Watanabe heritable hyperlipidemic (WHHL, ■) rabbit preparations (n=8,6,9) at 4, 6, and 12 months of age, respectively. Data points are means with SEM shown by vertical bars.
must be dilated to maintain normal cerebral blood flow. They claim that this is evidence for compensatory vasodilation by the intracranial arteries.

The later increased sensitivity of the basilar artery to histamine and NPY in preparations from 12-month-old animals, however, does not appear to be consistent with this hypothesis because their action would act against a compensatory vasodilator effect. It is possible that subsequent increased vasoconstriction of intracerebral blood vessels, even in the absence of obstructive plaque, could eventually lead to arterial spasm, ischemia, and stroke. In an extensive investigation performed by Hicks and Warren,10 it has been reported that in humans, cerebral vasospasm can occur without the presence of thrombus or mechanical occlusion. Furthermore, this vasospasm is local and not a result of a more general systemic circulatory failure, and they concluded that an intrinsic vascular change must be responsible.

Thus, it is possible that despite apparent protection of cerebral vessels from atherosclerotic lesions, such vessels may be affected by reduced blood flow due to occlusion of the extracranial (neck) arteries, which is compensated for by autoregulatory vasodilation. Potentiated endothelium-mediated responses, as measured in this study, would also contribute to an increase in vasodilation. However, in the longer term, changes leading to increased sensitivity to specific vasoconstrictor agents may explain the vasospasm, cerebral ischemia, and stroke that commonly occur in atherosclerosis.

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