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Potential role of angiotensin II in noise-induced increases in inner ear blood flow

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Guinea pigs were exposed to 120 dB white noise for 30 min and evidenced a four-fold elevation in plasma concentration of the potent vasoconstricting hormone angiotensin II (AII). Anesthetized animals received intra-arterial injections of AII at doses that approximated the endogenous levels measured following noise exposure. A marked decrease in skin blood flow was observed with a concomitant increase in cochlear blood flow as measured by laser Doppler flowmeters. Increased cochlear blood flow appeared to be secondary to the increases in systemic blood pressure induced by AII. These findings suggest that cochlear blood flow may increase during periods of intense noise exposure.

cochlear blood flow, angiotensin II, laser Doppler flowmeter, guinea pig

Introduction

Research concerned with the physiological mechanism underlying noise-induced hearing loss has focused on the role that vascular processes, particularly vasoconstriction, may play in mediating such loss and these efforts have yielded inconsistent findings [2]. Histological examination has indicated that noise-induced cochlear hair cell damage is associated with constriction of inner ear capillaries, and is correlated with decreased O_2 levels in inner ear endolymph during noise exposure. This suggests that noise-induced hearing loss is mediated, at least in part, by reduced blood flow of the cochlear vasculature which deprives inner ear structures of oxygen [8,17,20]. In contrast, Nuttall and co-workers [22] found no change in endolymph O_2 with short-duration noise exposure in anesthetized guinea pigs while direct observation of cochlear vessels during noise exposure by Perlman and Kimura [24] revealed increased cochlear blood flow. Prazma et al. [26] have also reported increased blood flow in the areas of the cochlea stimulated by noise. Furthermore, noise-induced temporary threshold shifts (TTS), which presumably contribute to permanent hearing loss [18], and peripheral vasoconstriction to loud noises, appear to be inversely related, i.e. greater peripheral vasoconstriction is associated with lesser degrees of TTS [5,10]. This suggests that changes in cochlear blood flow may depend at least partially upon changes in peripheral blood flow [29] and systemic blood pressure.

A potential mediator of such noise-induced blood flow changes is the blood-borne octapeptide angiotensin II (AII), a potent vasoconstrictor of vascular smooth muscle. A 25% reduction in renal blood flow has been observed in conscious rabbits during noise exposure [9]. This is a sufficient ischemia to stimulate the release of renin from the kidney which acts on circulating angiotensinogen to form AII [23]. Jugular vein infusion of AII (1 μg/kg) in anesthetized guinea pigs has been reported to cause a transient decrease in cochlear blood flow followed by an increase as measured by electrical impedance plethysmography [30]. How-

ever, this technique yields an indirect measure of blood flow and assumes, without confirmation, that external vessel caliber is proportional to flow. Our laboratory has measured significant elevations in circulating levels of AII in rodent and human subjects following exposure to loud noise [6,33]. Increases in plasma AII resulting from such exposure may thus modify cochlear blood flow either indirectly by shunting blood away from the peripheral vasculature or by acting directly upon the cochlear vasculature. The first mechanism should result in elevated cochlear blood flow concomitant with increased systemic blood pressure, while the second should produce decreased cochlear flow.

Cochlear microvascular anatomy has been elucidated [12,14,16,31], and cochlear blood flow has been measured using the microsphere technique [1]. Our research group has recently employed a helium–neon laser Doppler with a wavelength of 632 nm which is maximally sensitive to red blood cells (RBCs) to study some dynamic characteristics of inner ear blood flow [19]. This approach permits online measurement of blood flow through some cochlear vascular beds at a level of quantification not previously available. Laser Doppler probes were presently used to test three related questions. (i) Does exogenous AII induce cochlear blood flow changes? (ii) If so, in which direction relative to peripheral blood flow? (iii) Are the endogenous elevations in circulating levels of AII noted to occur following noise exposure sufficient to cause cochlear blood flow changes?

Methods

Mature female guinea pigs (Hartley, Charles River) were maintained in group cages under a 12 : 12 h light cycle initiated at 0700 h.

Noise exposure

The animals were divided into 3 groups of 6. Each member of the first group was exposed to white noise in a sound attenuation chamber. The noise originated from a General Radio random noise generator (Model 1382), was amplified by a MacIntosh 60 W amplifier (Model 240), and transduced by a JBL driver (Model 2482). The intensity of the sound at the animal's ear was adjusted to 120 dB re 20 μ Pa as calibrated with a Brüel & Kjaer sound level meter (Model 2209). Each animal was immobilized in a cloth wrapping with its head exposed, placed in the chamber, and subjected to the noise for 30 min. Within 30 s of noise termination each animal was decapitated and blood was collected in an ice-chilled, heparinized (25 U sodium heparin) centrifuge tube. The whole blood was immediately centrifuged at $2000 \times g$ for 15 min at 4°C to obtain the plasma fraction which was stored at −20°C until assayed for AII concentration. The second (control) group was treated identically; however, the noise was not presented.

Laser Doppler measurements

The 6 guinea pigs of the third group were anesthetized with 30 mg Ketamine and 4 mg Xylazine i.m. with supplemental injections when required (20 : 1 solution Ketamine : Xylazine). Each animal was prepared with a head post attached to parietal and frontal bone anchor screws with dental acrylic. The animal was also prepared with tracheal (PE 320, Clay Adams) and femoral artery (Type MRE-040, micro-renathan, Braintree Scientific) cannulae. The bulla was opened to expose the cochlea and the mucosa overlying the lateral wall of the basal turn of the cochlea was gently removed allowing the 'needle' probe (O.D. 1.75 mm) of a laser Doppler flow meter (Medpacific, LD-5000) to be positioned on the bony surface of the lateral wall of the promontory of the cochlea via a micromanipulator. Studies in skin suggest that this measure is sensitive to flow in vessels within 1 mm of the probe surface. In the guinea pig cochlea this would include primarily the lateral wall vessels. A second Doppler, prepared with a standard acrylic probe, was secured to a shaved portion of the animal's abdomen to permit measurement of skin blood flow. Blood pressure was monitored via the femoral artery cannula using a Gould-Statham transducer (Model P23Db) and a Sanborn polygraph (Model 850). Baseline measurements were obtained for blood pressure, cochlear and abdominal skin blood flows. Dose–response curves were then collected following the infusion of a 0.25 ml bolus of angiotensin II (U.S. Biochemicals) via the femoral artery cannula. Doses of 0.1, 1, 10 and 100 pM/kg in 0.15 M NaCl were administered in either ascending or descending order. Sufficient time was allowed between

injections to re-establish baseline values. For a more complete description of the laser Doppler and the theory underlying its use to measure blood flow changes of vascular beds refer to [7,19,21].

AII radioimmunoassay

The plasma of groups 1 and 2 was analyzed for AII levels in triplicate, 200 μl per assay. The plasma was initially extracted in 750 μl of 95% ethanol by incubation at 27°C for 15 min, then at 4°C for 15 min. The mixture was centrifuged at 2000 × *g* for 10 min at 4°C, and the supernatant was dried down overnight in a speedvac. Internal standards were used to assess the efficiency of extraction which averaged 84%. Values were normalized based on calculated recoveries. All glassware and assay tubes were coated with heat treated 0.1% BSA solution (Sigma 44503, 6 h at 56°C) and dried overnight prior to use. The incubation mixture contained the following components: 3 pg of [^{125}I]AII with a specific activity of 1700 μCi/μg in 200 μl of buffer (10 mM $NaPO_4$, pH 7.0); 100 μl of reconstituted rabbit antiserum diluted 1 : 25000 in buffer; 200 μl of standard AII or unknown; an additional 500 μl of buffer was added to bring the total reaction volume to 1.0 ml. Following incubation for 24 h at 4°C, 500 μl of dextran-coated charcoal (charcoal: Fischer C-170, 12 mg/ml; dextran: Sigma D-1390, 1.2 mg/ml) were added to the incubation mixture. The resultant suspension was vortexed, allowed to stand for 5 min and centrifuged at 2000 × *g* for 10 min at 4°C and the supernatant containing the bound angiotensin was retained for counting (Beckman Gamma 500). A complete standard curve, non-specific binding in the absence of antibody (blank) and zero standard binding was determined with the set of assays. The percentage of labelled AII bound was graphed against the total AII in the assay to form the standard curve. A logit transformation of the standard curve was used to calculate the unknowns expressed as ng/ml of plasma. The assay was sensitive to 1.5 pg of AII.

Results

Noise exposure and AII

Noise exposure elevated plasma AII levels to a mean (± S.E.) of 1.08 ± 0.26 ng/ml as compared with 0.28 ± 0.04 ng in the nonexposed control group ($t = 3.05$, d.f. = 10, $P < 0.02$). To our knowledge these are the first measures of AII taken in guinea pigs, however, normal levels of plasma AII range from approximately 0.07 to 0.10 ng/ml in rats and about 0.12 ng/ml in gerbils [11,27,32]. Therefore, the no-noise control group of the present study revealed somewhat elevated AII levels probably due to the stress of immobilization. Noise exposure (100 dB for 15 min) has previously been shown to elevate plasma AII concentration to a mean of about 0.64 ng/ml in rats [33], thus the present mean level of 1.08 ng following 30 min of noise exposure at 120 dB is in keeping with expected values.

Cochlear blood flow

There were marked increases in cochlear blood flow with concomitant decreases in skin flow

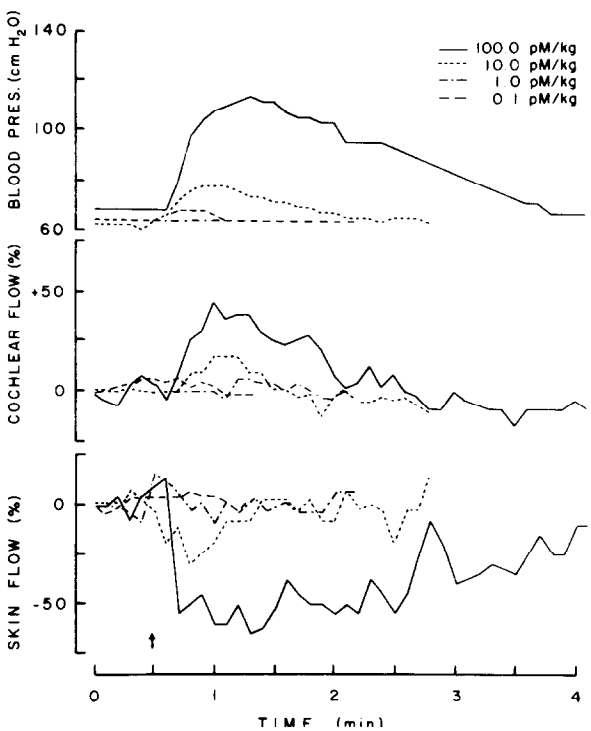

Fig. 1. Blood pressure, cochlear and abdominal skin blood flow changes induced by the injection of angiotensin II for animal No. 4 of group 3. The arrow indicates the time of the injection via the femoral artery cannula. The doses were administered in a descending order of magnitude with sufficient time (range 10–35 min) between doses to re-establish baseline values.

TABLE I

BLOOD PRESSURE, COCHLEAR AND ABDOMINAL SKIN BLOOD FLOW CHANGES FOLLOWING INTRA-ARTERIAL INJECTION OF ANGIOTENSIN II

Values are means ± S.E.

Dosage of AII	Blood pressure (cm H_2O)	Cochlear flow (%)	Skin flow (%)
0.1 pM/kg			
Max. change from baseline	+5.0±1.8	+3.6±0.9	−1.9±1.5
Baseline [a]	51.7±4.8	47.7±4.5	32.7±3.8
1.0 pM/kg			
Max. change from baseline	+6.8±2.6	+3.7±0.6	−4.3±2.8
Baseline	55.4±3.2	47.3±5.5	29.8±4.5
10 pM/kg			
Max. change from baseline	+13.4±2.8	+10.0±3.1	−7.2±2.3
Baseline	58.0±5.6	52.7±4.9	33.9±4.3
100 pM/kg			
Max. change from baseline	+44.8±1.4	+20.0±2.8	−12.8±3.2
Baseline	56.6±3.8	48.0±6.4	29.8±3.9

[a] Baseline measures were taken during the 30 s period preceding AII injection

coincident with AII injection. These blood flow dynamics followed a dose–response relationship and occurred in association with increases in systemic blood pressure. Table I summarizes the changes observed in each measure as a function of dose for all members of group 3. Fig. 1 presents representative results from a single animal of group 3. It should be observed that the laser Doppler provides a linear measure of blood flow as confirmed by other blood flow measurement techniques, including xenon clearance, microspheres and plethysmography [19]; however, the output is relative and not quantifiable in physical units of flow. Therefore, the data are presented as percent change from average baseline level in Table I and from average baseline expressed as 0 in Fig. 1.

Each data set was submitted to a Subjects × Dose repeated measures analysis of variance with further post-hoc analyses by Newman–Keuls test at $P = 0.05$ [3]. There were no differences in baseline blood pressure prior to the injection of each AII dose. There were differences among treatments with respect to maximum change in blood pressure ($F = 94.88$, d.f. $= 3/15$, $P < 0.0001$) in the expected direction, i.e. higher doses of AII resulted in greater elevations in blood pressure. The reader is cautioned that the baseline blood pressures were somewhat low and may indicate a compromised sympathetic nervous system. The maximum cochlear blood flow changes from baseline were significantly different among the doses ($F = 10.78$, d.f. $= 3/15$, $P < 0.001$) with larger elevations observed as dose increased. Abdominal skin blood flow exhibited the opposite pattern ($F = 3.89$, d.f. $= 3/15$, $P < 0.05$), i.e. larger decreases were observed as AII dose was increased. The 10 and 100 pM/kg doses were significantly different from each other and from the two lowest doses which did not differ.

Discussion

The present investigation initially determined that significant elevations in the vasoconstricting hormone, angiotensin II, occurred during intense noise exposure in the conscious guinea pig. These endogenous levels were then approximated by exogenous infusion of AII in anesthetized guinea pigs in order to measure changes in cochlear blood flow by laser Doppler probe. As a means of confirming that the infused doses of AII approximated those levels measured following noise exposure we estimated total plasma volume to be 4.5% of body weight [25]. The 10 and 100 pM/kg

doses infused into the animals of group 3 yielded concentrations of approximately 0.23 and 2.30 ng/ml, respectively. Since these values bracketed the mean plasma AII concentrations observed in the noise exposed animals of group 1, i.e. 1.08 ng/ml, the skin and cochlear blood flow dynamics observed following the two highest doses of AII in group 3 may reflect those that occurred in the noise exposed animals. The present laser Doppler results complement an earlier report by Prazma et al. [26] which indicated elevated cochlear blood flow in the stria vascularis and basilar membrane of the first turn stimulated by high-intensity white noise (10–42 kHz). Blood flow in the nonstimulated upper turns did not change. Thus, cochlear blood flow appears to be influenced not only by systemic blood pressure as presently observed, but also by local mechanisms. Our results are also in agreement with a recent report indicating that AII infusion in the dose range presently used, caused vasoconstriction of mesenteric and renal vascular beds in the alert, free-moving rat as measured by sonar Doppler probes [13]. However, there is the possibility in the present investigation that lowered blood pressure resulting from anesthetization compromised the animal's ability to evidence cochlear autoregulation and therefore flow would become proportional to blood pressure [9].

The issue of anesthetization induced differences in responding to noise and vasoactive compounds due to altered sympathicotone is most relevant to the present results concerning laser Doppler measurement of cochlear flow. At the present state of development, laser Doppler probes are much too large to implant chronically in order to measure cochlear flow in the alert animal. Sonar Doppler probes can be implanted [13]; however, these probes lack the specificity necessary to record from discrete capillary beds.

In summary, these data indicate that (i) it is possible to use a laser Doppler needle probe to monitor cochlear vascular blood flow changes in the anesthetized animal; (ii) noise-induced elevations in endogenous AII are of sufficient magnitude to significantly affect cochlear blood flow; (iii) exogenously administered AII appears to increase cochlear flow; and, coupled with the findings of Lappe and Brody [13], (iv) the increased cochlear blood flow is probably secondary to the elevation in systemic blood pressure accompanying vasoconstriction of mesenteric, renal and peripheral skin vascular beds. It would appear that the observed decreased endolymph pO_2 reflects an increase in endorgan activity and metabolism [28], which the increased blood flow is unable to accommodate. This notion is supported by two recent reports. Canlon and Schacht [4] have measured increased glucose utilization with noise exposure in the organ of Corti and tissues of the lateral wall of the mouse following deoxy[^{3}H]glucose injection. LeDoux and co-workers [15] have indicated that brief presentation of a pure tone at 80 dB increased blood flow in the central auditory pathway as measured by the ^{14}C-labeled iodoantipyrine technique. Thus, noise-induced elevations in blood flow may occur within the capillaries in different regions of the auditory system. However, confirmation of these findings in the alert animal will be necessary before the question can be settled whether the cochlear blood flow during acute noise is indeed increased.

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References

1 Angelborg, C., Hultcrantz, E. and Ågerup, B. (1977): The cochlear blood flow. Acta Otolaryngol. 83, 92–97.

2 Axelsson, A. and Vertes, D. (1982): Histological findings in cochlear vessels after noise. In: New Perspectives on Noise-Induced Hearing Loss, pp. 49–68. Editors: R.P. Hamernik, D. Henderson and R. Salvi. Raven Press, New York.

3 Bruning, J.L. and Kintz, B.L. (1977): Computational Handbook of Statistics, pp. 44–48. Scott, Foresman and Co., Glenview, Ill.

4 Canlon, B. and Schacht, J. (1983): Acoustic stimulation alters deoxyglucose uptake in the mouse cochlea and inferior colliculus. Hearing Res. 10, 217–226.

5 Dengerink, J.E., Dengerink, H.A. and Chermak, G.D. (1982): Personality and vascular responses as predictors of temporary threshold shifts after noise exposure. Ear Hearing 3, 196–201.

6 Dengerink, H.A., Wright, J.W., Thompson, P. and Dengerink, J.E. (1982): Changes in plasma angiotensin II with noise exposure and their relationship to TTS. J. Acoust. Soc. Am. 72, 276–278.
7 Goodwin, P.C., Miller, J.M., Dengerink, H.A., Wright, J.W. and Axelsson, A. (1984): The laser Doppler: A non-invasive measure of cochlear blood flow. Acta Otolaryngol. (in press).
8 Hawkins, J.E. (1971): The role of vasoconstriction in noise-induced hearing loss. Ann. Otolaryngol. 80, 903–913.
9 Hultcrantz, E. (1979): The effect of noise on cochlear blood flow in the conscious rabbit. Acta Physiol. Scand. 106, 29–37.
10 Jansen, G. (1969): Relation between temporary threshold shift and peripheral circulatory effects of sound. In: Physiological Effects of Noise, pp. 667–741. Editors: B.L. Welch and A.M. Welch. Plenum Press, New York.
11 Johnson, A.K., Mann, J.F.E., Rascher, W., Johnson, J.D. and Ganten, D. (1981): Plasma angiotensin II concentrations and experimentally induced thirst. Am. J. Physiol. 240, R229–R234.
12 Kimura, R.S. and Schuknecht, H.F. (1979): The ultrastructure of the human stria vascularis, Part II. Acta Otolaryngol. 70, 301–318.
13 Lappe, R.W. and Brody, M.J. (1984): Mechanisms of the central pressor action of angiotensin II in conscious rats. Am. J. Physiol. 246, R56–R62.
14 Lawrence, M. (1980): Control mechanisms of inner ear microcirculation. Am. J. Otolaryngol. 1, 324–334.
15 LeDoux, J.E., Thompson, M.E., Iadecola, C., Tucker, L.W. and Reis, D.J. (1983): Local cerebral blood flow increases during auditory and emotional processing in the conscious rat. Science 221, 576–578.
16 Maass, B. (1981): Autonomic nervous system and hearing. Adv. Oto-Rhino-Laryngol. 27, 14–25.
17 Maass, B., Baumgartl, H. and Lubbers, D.W. (1976): Local PO_2-measurements and PH_2-measurements with needle electrodes and examination of cochlea. Arch. Oto-Rhino-Laryngol. 214, 109–124.
18 Melnick, W. (1978): Temporary and permanent threshold shifts. In: Noise and Audiology, pp. 83–107. Editor: D.M. Lipscomb. University Park Press, Baltimore.
19 Miller, J.M., Marks, N.J. and Goodwin, P.C. (1983): Laser Doppler measurements of cochlear blood flow. Hearing Res. 11, 385–394.
20 Misrahy, G.A., Shinabarger, E.W. and Arnold, J.E. (1958): Changes in cochlear endolymphatic oxygen availability, action potential, and microphonics during and following asphyxia, hypoxia, and exposure to loud sounds. J. Acoust. Soc. Am. 30, 701–704.
21 Nilsson, G.E., Tenland, T. and Oberg, P.A. (1980): Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE Trans. Bio-Med. Eng. 27, 597–604.
22 Nuttall, A.L., Hultcrantz, E. and Lawrence, M. (1980): Does loud sound influence the intracochlear oxygen tension? Hearing Res. 5, 285–293.
23 Peach, M.J. (1977): Renin-angiotensin system: Biochemistry and mechanisms of action. Physiol. Rev. 57, 313–370.
24 Perlman, H.B. and Kimura, R.A. (1955): Observations of the living blood vessels of the cochlea. Ann. Otolaryngol. 64, 1176–1192.
25 Pitts, R.F. (1968): Physiology of the Kidney and Body Fluids, Vol. 22. Year Book Medical Publishers, Chicago.
26 Prazma, J., Rodgers, G.K. and Pillsbury, H.C. (1983): The effect of noise on cochlear blood flow. Presented at the ARO Midwinter Meeting, 23–27 January, St. Petersburg, Fla.
27 Russell, P.J.D., Abdelaal, A.E. and Mogenson, G.J. (1975): Graded levels of hemorrhage, thirst and angiotensin II in the rat. Physiol. Behav. 15, 117–119.
28 Ryan, A.F., Goodwin, P.C., Woolf, N.K. and Sharp, F. (1982): Auditory stimulation alters the pattern of 2-deoxyglucose uptake in the inner ear. Brain Res. 234, 213–225.
29 Snow, J.B. and Suga, F. (1972): Control of cochlear blood flow. In: Vascular Disorders and Hearing Defects, pp. 167–183. Editor: J.D. Delorenzo. University Park Press, Baltimore.
30 Suga, F. and Snow, J.B. (1969): Cochlear blood flow in response to vasodilating drugs and some related agents. Laryngoscope 79, 1956–1979.
31 Vertes, D., Axelsson, A. and Lipscomb, D.M. (1979): Some vascular effects of noise exposure in the chinchilla cochlea. Arch. Otorhinolaryngol. 224, 97–101.
32 Wright, J.W. and Harding, J.W. (1980): Body dehydration in xeric adapted rodents: Does the renin–angiotensin system play a role? Comp. Biochem. Physiol. 58A, 137–142.
33 Wright, J.W., Dengerink, H.A., Thompson, P. and Morseth, S. (1981): Plasma angiotensin II changes with noise exposure at three levels of ambient temperature. J. Acoust. Soc. Am. 70, 1353–1356.