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Acoustic stimulation alters deoxyglucose uptake in the mouse cochlea and inferior colliculus

Barbara Canlon and Jochen Schacht

Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109, U.S.A.

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Deoxyglucose uptake and activities of hexokinase and glucose-6-phosphatase in auditory structures (organ of Corti, stria vascularis and spiral ligament, modiolar section of VIIIth nerve, inferior colliculus) and non-auditory tissues (heart, kidney, liver) of the mouse were analyzed. [^{3}H]Deoxyglucose was given as a pulse into the tail vein and uptake was quantitated by microdissection of tissues and scintillation counting. Radioactivity in cochlear tissues was maximal after 45–60 min and declined with a half-life of 30–60 min. Deoxyglucose 6-phosphate represented ca. 60% of total radioactivity (heart, inferior colliculus, > 80%). The ratio of hexokinase to glucose-6-phosphatase activity was considerably lower in the auditory periphery than in brain. The rank order was inferior colliculus ≫ VIIIth nerve ≈ heart > stria vascularis and spiral ligament > kidney > organ of Corti ≈ liver.

Exposure to broadband noise increased glucose utilization in all auditory structures. Uptake was maximally (2- to 3-fold) stimulated at moderate noise intensity (55–85 dBA). In addition, the auditory system showed two salient features: at high intensities (100 and 115 dBA) deoxyglucose uptake decreased from the maximum; and the non-sensory tissues of the cochlea (stria vascularis and spiral ligament) responded to sound parallel to the sensory structures at all levels of stimulus intensity. There were no effects of acoustic stimulation on serum glucose levels, serum kinetics of deoxyglucose, or deoxyglucose uptake into other body tissues.

Key words: deoxyglucose; auditory structures; energy metabolism; acoustic stimulation; noise.

Introduction

Biochemical correlates of acoustic transduction have yet to be established for the peripheral auditory system. Paradoxically, a wide variety of noise-induced changes in the tissues and fluids of the inner ear have been reported without providing a basis for an understanding of sound processing. Effects on DNA and RNA, protein synthesis, enzymatic activities, permeability and transport processes may reflect various stages in noise-induced trauma rather than molecular events related to auditory transduction. Quantitative biochemical studies, notably on high-energy metabolites, have failed to demonstrate effects of non-traumatic exposure to noise (for reviews, see [6,21,24]).

Since its introduction by Sokoloff et al. [23], the technique of 'deoxyglucose trapping' has been widely used to investigate the metabolic response of the central

nervous system to physiological stimuli. In the present study we adapted the procedure for the auditory periphery to investigate deoxyglucose uptake in the cochlea and the inferior colliculus of the mouse in response to acoustic stimulation.

Materials and Methods

Deoxyglucose uptake

Conscious male mice (CBA, Charles River Laboratories), 4–12 weeks of age (16–25 g), were injected with a single pulse of 5 mCi 2-deoxy-D-1-[^{3}H]glucose (Amersham S.A., 40 mCi/mmol)/kg body wt in the tail vein (in approx. 0.2 ml saline per injection). Immediately after the injection the animals were placed into a sound-shielded 'exposure box'. At various time intervals animals were killed, blood samples taken and the cochlea, inferior colliculus, kidney, liver and heart removed. The organs were quickly blotted on tissue paper to remove adhering blood and exposed to microwave irradiation (1200 W) for 15 s to arrest enzymatic activities. Prior to microwaving the cochlea, the inner ear fluids were absorbed with a cotton swab. Tissues were homogenized and aliquots taken for protein determination and scintillation counting. Aliquots were also processed for separation of deoxyglucose from deoxyglucose 6-phosphate by ion-exchange chromatography [9].

Dissection of the inner ear

The inner ear was hand dissected under a microscope into three components: (1) the 'organ of Corti' consisting of receptor cells, supporting cells and nerve fibers; (2) the lateral wall tissues, a combined preparation of the stria vascularis and spiral ligament; and (3) the modiolar portion of the VIIIth nerve.

Sound exposure

The exposure box ($40 \times 40 \times 40$ cm) had loud speakers mounted in the ceiling and was placed inside a soundproof room in order to obtain the lowest noise levels (25 dBA, 42 dBB, 56 dBC). Auditory stimulation was broadband noise (100 Hz to 45 kHz) at intensities of up to 115 dBA. The spectrum was flat for frequencies up to 20 kHz and attenuated approx. 20 dB between 20 and 45 kHz. Noise levels were measured with a half-inch condensor microphone on the dBA scale.

Biochemical assays

Hexokinase (ATP:D-hexose-6-phosphotransferase, EC 2.7.1.1) and glucose-6-phosphatase (glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) were analyzed with the radioactive substrates, [^{3}H]deoxyglucose and [^{3}H]deoxyglucose 6-phosphate, respectively. Deoxyglucose was obtained from Sigma (St. Louis, Mo., U.S.A.) and [^{3}H]deoxyglucose 6-phosphate was synthesized enzymatically [3]. Tissues were homogenized in 0.25 M sucrose/5 mM potassium phosphate, pH 7.6, and centrifuged for 10 min at $12000 \times g$. The supernatant fraction was assayed for hexokinase in (final concentrations) 40 mM potassium phosphate, pH 7.6, 40 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 0.1 mM (10 nCi) deoxyglucose, total volume, 100 μl. The pellet

was suspended in 50 mM sodium cacodylate, pH 6.5, and assayed for glucose-6-phosphatase with 0.1 mM (10 nCi) deoxyglucose 6-phosphate, volume 100 μl [7].

Protein [14] and glucose [2] were determined spectrophotometrically.

Calculations

All values of deoxyglucose uptake were calculated from the radioactivity in the tissue per μg tissue protein and corrected for serum levels of [^{3}H]deoxyglucose. Serum glucose levels were not taken into account as they did not change with noise exposure. Furthermore, a 'lumped constant' necessary for the calculation of absolute metabolic rates [23] cannot be obtained for individual cochlear tissues. Even if it were technically feasible to measure arterio-venous differences between the labyrinthine artery and the vein of the cochlear aqueduct, these would only reflect the combined metabolism of the whole cochlea and the vestibular system.

Results

Deoxyglucose uptake

Deoxyglucose uptake in the organ of Corti, lateral wall tissues, and VIIIth nerve was maximal between 45 and 60 min (Fig. 1), when the ratio of deoxyglucose to deoxyglucose 6-phosphate had also reached an equilibrium [4]. A rapid decline of radioactivity thereafter indicated a short half-life of the radioactive compounds in

TABLE I

PHOSPHORYLATION OF [^{3}H]DEOXYGLUCOSE AND ENZYMATIC ACTIVITIES IN MOUSE TISSUES

Phosphorylation (in vivo): Animals received a pulse of 5 mCi [^{3}H]deoxyglucose/kg body wt and were killed at 60 min. Tissues were analyzed as described in Methods and deoxyglucose 6-phosphate was separated by ion-exchange chromatography [9]. There was no difference of phosphorylation with varying noise intensities. Numbers are means ± S.D. with number of animals in parentheses. Enzymatic activities (in vitro) were determined from initial rates (1–3 or 2–5 min) as described in Methods. Ratios are taken from means of duplicate experiments.

Tissue	Deoxyglucose 6-phosphate (% of total radioactivity)	Enzyme activities (Hexokinase/ glucose-6-phosphatase)
Organ of Corti	56 ± 10 (28)	1.6
Lateral wall tissues	63 ± 9 (28)	3.5
Inner ear fluids	10 ± 7 (5)	– [a]
VIIIth nerve	76 ± 8 (15)	6.5
Inferior colliculus	84 ± 4 (12)	14.3
Heart	82 ± 5 (18)	5.5
Kidney	47 ± 9 (13)	2.2
Liver	29 ± 14 (7)	1.5

[a] Not determined.

the inner ear tissues. In the organ of Corti deoxyglucose 6-phosphate comprised 55% of total radioactivity, in the lateral wall tissues 63% and in VIIIth nerve and inferior colliculuc about 80% (Table I). For comparison, heart showed a conversion to the phosphate of 82%, while kidney and liver were lowest (approx. 40%). Inner ear fluids contained almost no phosphorylated product.

Enzymatic activities

The activities of hexokinase and glucose-6-phosphatase of cochlear tissues were compared to those of the inferior colliculus, kidney, heart and liver. Assays were carried out with 25–50 μg of protein for 3–5 min since under these conditions enzymatic rates were linear with respect to both time and protein. Activities for inferior colliculus were typical for structures of the central nervous system, high hexokinase and low phosphatase activity [7]. In contrast, cochlear tissues had lower hexokinase levels and higher phosphatase activities (Table I).

Effects of acoustic stimulation

Noise exposure increased the levels of deoxyglucose in all auditory tissues (Fig. 1). Since the 60 min time point showed optimal incorporation, phosphorylation, and a clearly established effect of noise, it was chosen for subsequent experiments (Fig. 2). Deoxyglucose uptake in both the cochlear tissues and the inferior colliculus increased two- to three-fold with auditory stimulation of moderate intensity (55–85 dBA). At higher intensities (100 and 115 dBA) the rate of glucose utilization decreased but still remained somewhat higher than at rest. Radioactivity in inner ear fluids apparently did not follow this pattern. After corrections for inter-animal differences in serum radioactivity the fluids contained 165 ± 45 dpm at

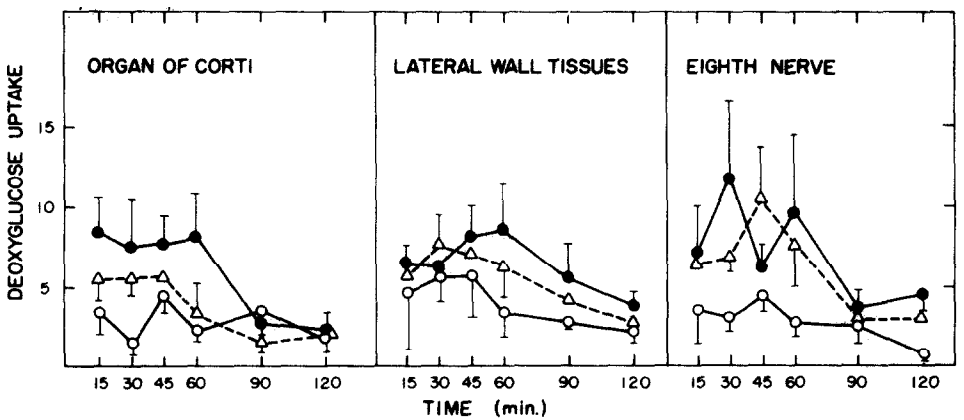

Fig. 1. Time course of deoxyglucose uptake into cochlear tissues. Animals received a pulse of 5 mCi [^{3}H]deoxyglucose/kg body weight and were killed at times indicated. Deoxyglucose uptake was determined as described in Methods. Values are means ± S.D. for 3 to 6 animals each. ○, no sound exposure; ●, exposure to 45 dBA; △, exposure to 100 dBA white noise during the time of the experiment. CB57/J mice (6–12 weeks old) were used.

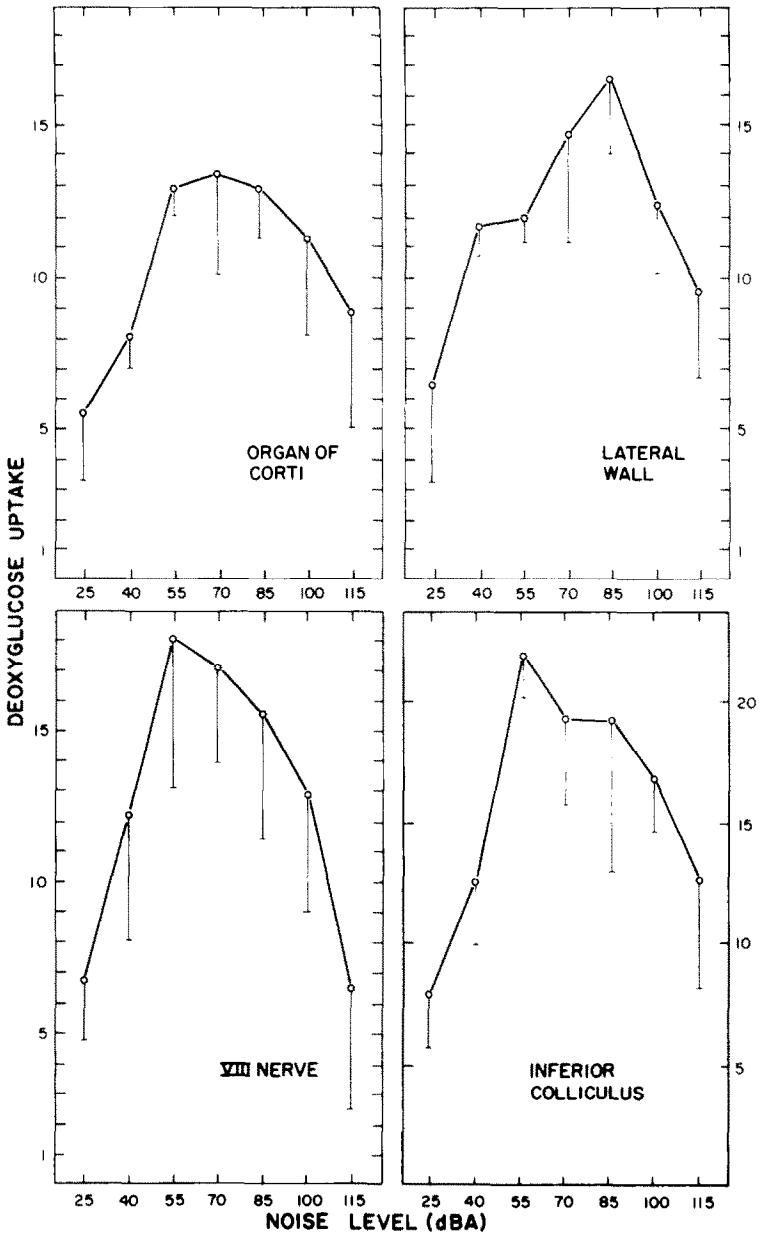

Fig. 2. Response of deoxyglucose uptake to noise exposure. Animals received a pulse of 5 mCi [^{3}H]deoxyglucose/kg body wt and were killed after 60 min of exposure to the noise levels indicated. Deoxyglucose uptake was determined as described in Methods. Values are means $\pm$ S.D. from 3 to 8 animals each. Differences between 25 dBA and 85 dBA and between 85 dBA and 115 dBA are significant at $P < 0.001$ for all tissues.

25 dBA ($n = 7$), 222 ± 110 dpm at 85 dBA ($n = 5$) and 244 ± 127 dpm at 115 dBA ($n = 4$).

Several parameters were tested to determine possible systemic responses to noise exposure. Kinetics of [^{3}H]deoxyglucose in the serum remained unaltered by noise exposure as did serum glucose levels. This was established both for early times (to 60 s) where the peak of serum radioactivity occurred and for later times corresponding to the conditions of sound exposure (Fig. 3).

Moreover, glucose utilization in the renal cortex, liver and heart appeared to be

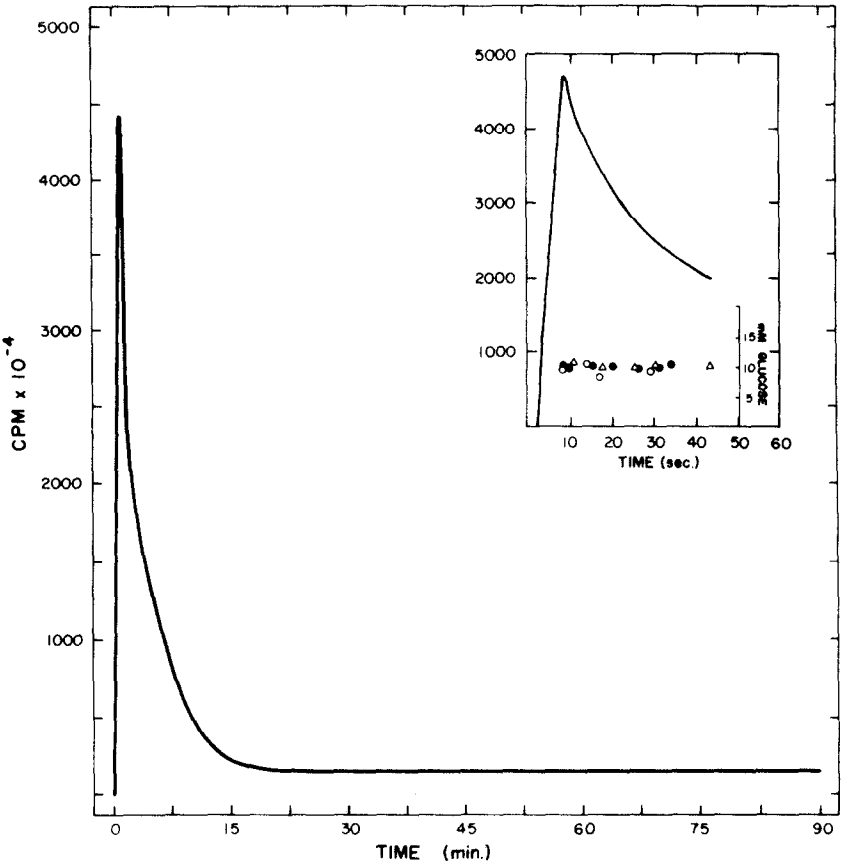

Fig. 3. Time course of serum radioactivity and glucose levels. Animals received a pulse of 5 mCi [^{3}H]deoxyglucose/kg body weight and were exposed to noise as described in Methods. For radioactivity, 58 serum samples were analyzed for the time of 2–60 s after exposure ($n = 16$ at 25 dBA, $n = 24$ at 85 dBA, $n = 18$ at 115 dBA); at later times (15, 30, 45, 60 and 90 min) 3–6 samples were analyzed at each dB level. Serum kinetics did not differ with noise exposure ($P > 0.5$; two-way ANOVA). Data from all sound exposures was combined to construct the curves by polynomial best-fit. Glucose values are from individual samples at 25 dBA (○), 85 dBA (●) and 115 dBA (△). Serum glucose levels at 60 min were (means ± S.D. from five animals per group): 10.0 ± 2.4 mM (25 dBA), 11.5 ± 1.1 mM (85 dBA), and 11.3 ± 0.8 mM (115 dBA).

TABLE II

EFFECT OF ACOUSTIC STIMULATION ON DEOXYGLUCOSE UPTAKE IN NON-AUDITORY TISSUES

Animals received a pulse of 5 mCi [^{3}H]deoxyglucose/kg body weight and were killed after 60 min. Deoxyglucose uptake was determined as described in Methods. Numbers are means ± S.D. with number of animals in parentheses.

Tissue	Deoxyglucose uptake		
	25 dBA	85 dBA	115 dBA
Liver	2.2 ± 0.5 (9)	3.4 ± 1.9 (11) *	1.9 ± 0.4 (7)
Kidney	4.5 ± 2.3 (8)	7.1 ± 3.3 (11) **	4.5 ± 2.8 (7)
Heart	16.5 ± 5.2 (3)	14.1 ± 4.9 (3)	19.1 ± 10.3 (3)

Differences between 85 dBA and 25 dBA (one-way ANOVA): * $0.10 > P > 0.09$; ** $0.08 > P > 0.07$.

independent of the auditory stimulus (Table II). However, since the somewhat elevated deoxyglucose levels in kidney and liver had P values between 0.05 and 0.1, the data were more closely inspected. Further analysis showed that of the 11 animals exposed to 85 dBA, 7 gave a cluster of values indistinguishable from controls at 25 dBA (4.9 ± 1.4 for kidney; 2.2 ± 0.6 S.D. for liver). Two animals each had higher uptake into either kidney or liver, and only two animals showed increased uptake into both kidney (13.1 and 9.7) and liver (6.1 and 4.5). In addition, results from two small groups of animals ($n = 4$) exposed to 70 or 100 dBA did not differ significantly from control values. In contrast, all values in all stimulated (85 dBA) cochlear tissues were higher than all corresponding control values and the significance of these differences was $P < 0.001$.

Discussion

Glucose utilization in the inner ear exhibited two salient features: (1) a biphasic response to the sound stimulus, and (2) a response to sound of the non-sensory tissues of the lateral wall of the cochlea. Before the significance of these findings can be assessed, however, it seems indicated to discuss the application of the deoxyglucose technique to the inner ear.

Deoxyglucose 'trapping' in the cochlea differs from that in brain in that the ratio of deoxyglucose 6-phosphate to deoxyglucose is considerably lower and the half-life of radioactivity is shorter. Such results are suggestive of a high glucose-6-phosphatase activity in the organ of Corti and the tissues of the lateral wall as confirmed by direct enzymatic analysis. The rank order of activities in brain (high hexokinase, low phosphatase), kidney and liver (low hexokinase, high phosphatase) is in good agreement with previously reported enzymatic activities on 2-deoxy-2-fluoro-D-glucose [7]. The enzymatic pattern of the cochlear tissues is apparently closer to body tissues such as kidney or liver than to the central nervous system. High glucose-6-

phosphatase activity may cause dephosphorylation and loss of deoxyglucose post-mortem from tissues where surgical access is difficult and time consuming as in the cochlea. Our technique of arresting enzymatic activities by microwave irradiation and microdissection of the tissues should minimize artifacts.

It is interesting that deoxyglucose uptake was maximally stimulated at physiological levels of sound (55–85 dBA). The effects of noise can only be observed when control animals are completely shielded from ambient noise confirming for the cochlea what had been demonstrated for central auditory pathways [22]. Comparison with previous reports of deoxyglucose trapping in the inner ear is difficult because of the use in those studies of tissues embedded for light or electron microscopy [18,19,20]. Such preparations may retain only a fraction of the trapped deoxyglucose and of this a significant amount may be associated with glycogen granules [12,18]. In their radioautographic study in the gerbil, Ryan et al. [19] observed sound-stimulated deoxyglucose uptake in the VIIIth nerve but not in stria vascularis and only marginally in the organ of Corti. Not only the differences in the analytical methods but also in the sound stimulus and species preclude a direct comparison with our data.

Noise, particularly at high intensities, can cause stress to an animal [16]. It appears, however, that general systemic responses do not influence deoxyglucose trapping in the inner ear. Serum glucose levels and kinetics of [^{3}H]deoxyglucose remain unchanged over the intensities given. Furthermore, deoxyglucose uptake into non-auditory tissues is not dependent on acoustic stimulation. Therefore, the observed changes seem specific for the auditory structures.

A striking feature of glucose utilization in the auditory periphery is the decrease at high stimulus intensities, the reasons for which remain speculative. Sound-induced increase in hair cell permeability has been reported [7] but we do not find the increased radioactivity in the inner ear fluids that would be expected if such a change were occurring. Moreover, since VIIIth nerve and inferior colliculus follow the pattern of the cochlear tissues, a genuine stimulus-related phenomenon is suggested. A somewhat similar phenomenon of decreased glucose utilization at high stimulus intensities has been reported for the retina [15] and damage to retinal rods was suggested as the cause. The decrease we observe in the cochlea could similarly result from injury to hair cells and represent a noise-induced threshold shift. Hair cell damage due to noise trauma is well documented [11] but highly variable with exposure conditions and animal species. Preliminary results from our laboratory indicate that exposure to 115 dBA for 60 min does not induce permanent metabolic damage to the cochlea.

It should be considered that a biphasic response to sound stimulation can also be observed in electrophysiological responses of the ear. For instance, the cochlear microphonic potential rises with stimulus intensity of pure tones and decreases with overstimulation [24]. While differences in experimental parameters (pure tones of short duration in anesthetized animals in electrical recordings; continuous broadband noise and conscious animals in our study) do not permit direct comparisons it is nevertheless possible that the same mechanisms underly the electrophysiological and metabolic observations. These might include the stapedius reflex, efferent

inhibition or adaptation [5,17]. More likely, a reduction in local blood flow and consequent ischemia could be responsible for the decrease. Vasoconstriction of cochlear vessels in response to sound of high intensities has been reported [10] but remains controversial [1]. Morphological and histological investigations under the conditions of our experiments are needed to test these hypotheses.

The other salient finding of our study is the response of the non-sensory tissues of the cochlea to acoustic stimulation. The stria vascularis and the spiral ligament do not receive innervation, yet their deoxyglucose uptake is stimulated parallel with that of organ of Corti, VIIIth nerve and inferior colliculus. The functional role of the stria vascularis is presumed to be the maintenance of the endolymphatic potential, a resting potential crucial for the function of the auditory end-organ. Electrical activity of the receptor cells in the organ of Corti could alter potassium or sodium fluxes in the endolymph and thus provide an electrical feedback from the hair cells to the stria vascularis. Another explanation for the coupling of metabolism between the lateral wall tissues and the organ of Corti is the possible effect on blood flow of the sound stimulus. Control of blood flow in the cochlea may be located at the modiolar level where a vasomotor innervation is present [13]. Thus, if both the increase and decrease of glucose utilization are paralleled by increases and decreases of cochlear blood flow, then the organ of Corti and the lateral wall tissues should be equally affected.

In summary, the increase of glucose utilization of the auditory periphery with moderate acoustic stimulation provides a good analogy to central auditory structures and their response to physiological stimuli. The specific features of glucose utilization in the cochlea, the decrease with high noise intensities and the response of the non-neural tissues require further investigation. The first phenomenon may represent an early stage of noise-induced trauma while the second may provide new insights into cochlear physiology.

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