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The Effect of Noise on Deoxyglucose Uptake into Inner Ear Tissues of the Mouse*

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Deoxyglukoseaufnahme in das Innenohrgewebe der Maus nach Lärmeinwirkung

Zusammenfassung. Die Aufnahme von Deoxyglukose in das Innenohrgewebe wurde bei Mäusen untersucht. Sechzig Minuten nach einmaliger i.v. Injektion von 5 mCi 2-Deoxy-D[^{3}H]-Glukose/kg Körpergewicht wurden Stria vascularis/Ligamentum spirale und Cortisches Organ sowie andere Körpergewebe seziert und ihre Radioaktivität gemessen. Die Aufnahme in das Innenohrgewebe war 3–5mal niedriger als in Herz oder Gehirn. Das Verhältnis von Deoxyglukose-6-Phosphat zu Deoxyglukose war 60 : 40, und die Substanzen wurden mit einer kurzen Halbwertszeit (ca. 60 min) aus dem Innenohr ausgeschieden. Beschallung mit white noise (100 dB) während des radioaktiven Pulses führte zu einer 50%igen Erniedrigung der Einbaurate in die Innenohrgewebe.

Schlüsselwörter: Deoxyglukose – Lärm – Innenohr

Summary. The uptake of deoxyglucose into inner ear tissues was studied in the mouse. Sixty minutes after a single i.v. injection of 5 mCi 2-deoxy-D-[^{3}H]glucose/kg body weight, stria vascularis/spiral ligament and organ of Corti as well as other body tissues were dissected and analyzed for radioactivity. Uptake into inner ear tissues was three to five times lower than into brain or heart. The ratio of deoxyglucose-6-phosphate to deoxyglucose was 60 : 40 and the compounds were eliminated from the inner ear with a half life of approximately 60 min. Exposure to 100 dB of white noise during the radioactive pulse decreased uptake of deoxyglucose into both stria vascularis/spiral ligament and organ of Corti by 50%.

Key words: Deoxyglucose – Noise – Inner ear

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Introduction

"Deoxyglucose trapping" is a non-invasive procedure for the measurement of energy metabolism. A pulse of radioactively labeled 2-deoxy-D-glucose is injected into the animal as a tracer for glucose. It is transported into the cell by the same carrier as glucose and phosphorylated by hexokinase to deoxyglucose-6-phosphate. This compound is not metabolized further by the enzymes of glucose metabolism and, as a charged species, is essentially trapped inside the cell. The amount of deoxyglucose-6-phosphate accumulated in the cell is directly proportional to the rate of glucose metabolism during the time of the radioactive pulse. In a tissue that exclusively utilizes glucose as its energy source, such as brain under normal conditions, the rate of the deoxyglucose accumulation is a direct reflection of energy metabolism.

The procedure has been adapted to measure glucose metabolism in specific regions of the brain and can be used to correlate local functional activity and energy metabolism. In the central nervous system (CNS) the auditory pathways are metabolically highly active (Sokoloff 1977) and different areas of the cochlear nucleus respond to stimuli of different frequencies with increases in glucose metabolism (Webster et al. 1978).

While the deoxyglucose method as a measure of energy metabolism has been validated for the brain (Sokoloff et al. 1977) and also for the heart (Gallagher et al. 1978), it is not equally applicable to other organs such as liver and kidney (Gallagher et al. 1978). High levels of glucose-6-phosphatase, which is virtually absent from brain, can significantly dephosphorylate deoxyglucose-6-phosphate which then diffuses out of the cell. Another difficulty in interpreting results of deoxyglucose trapping is encountered in organs which store large quantities of glycogen. Energy metabolism of these tissues need not directly correspond to the measured glucose utilization.

We studied the effect of noise on deoxyglucose trapping in the cochlea by dissection of the inner ear tissues and quantitation of radioactivity by scintillation counting.

Methods

Conscious male mice (CBJ/57) weighing 15–22 g were injected with a single dose of 5 mCi 2-deoxy-D[1-^{3}H] glucose (The Radiochemical Center, Amersham, UK, 19.5 mCi/mmol)/kg body weight into the tail vein. After the injection, animals were individually kept in a sound proof box at an ambient noise level of approximately 30 dB (controls), or at 100 dB white noise. At various time intervals animals were killed, blood samples taken, and brain, heart, kidney, liver, and cochlea removed. The organs were quickly blotted on tissue paper to remove adhering blood and exposed to microwave irradiation for 15 s to arrest enzymatic activities. When individual cochlear tissues were to be dissected, the inner ear fluids were drained, prior to microwaving, by inserting a glass capillary tube via the round window. The tissues were then dissected in the dry state to prevent any diffusion of deoxyglucose. Tissues were homogenized and aliquots taken for protein determination (Lowry et al. 1951) and scintillation counting. Aliquots were also processed for separation of deoxyglucose from deoxyglucose-6-phosphate on a Dowex-1 anion exchange column (Hassell et al. 1975).

The uptake of radioactive deoxyglucose into the tissues was determined by correcting for the protein content in the tissues and for total serum levels of deoxyglucose. The serum levels were obtained at killing and were integrated for the time of the experiment on the basis of a previously determined function for deoxyglucose kinetics in serum.

Results

Uptake of ^{3}H-deoxyglucose into the whole cochlea of conscious animals was sufficiently high to permit analysis of individual tissues as well as analysis of the rate of conversion of deoxyglucose to deoxyglucose-6-phosphate. The final ratio of deoxyglucose-6-phosphate to deoxyglucose was reached after about 60 min and remained constant for the duration of the experiment (Fig. 1). Of the total radioactivity found in the cochlea, only about 60% represented deoxyglucose-6-phosphate, compared to a conversion of more than 90% in the heart (Fig. 1). Kidney showed a similar ratio of conversion as the cochlea (Fig. 1).

When the amounts of deoxyglucose-6-phosphate trapped in the cochlear tissues are considered (Fig. 2), a maximum appeared to be reached after about 60 min, which was followed by a rather steep decline of the amount of this compound indicating an elimination with a half-life of approximately 60 min.

The uptake of ^{3}H-deoxyglucose into the combined preparation of stria vascularis and spiral ligament or into the organ of Corti (Table 1) was less than into brain (400 cpm) or heart (1,160 cpm). It should be considered that the values for inner ear tissues may be overestimates since residual inner ear fluid adhering to the tissues would increase the amount of radioactivity found. Both the ratio of deoxyglucose-6-phosphate to deoxyglucose and the half-life of deoxyglucose-6-phosphate were similar to the whole cochlea described above.

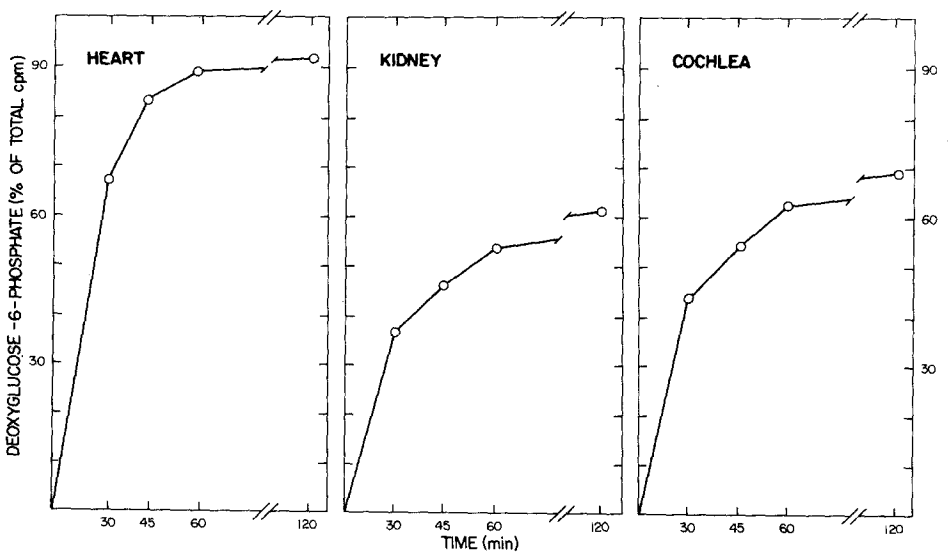

Fig. 1. Phosphorylation of deoxyglucose in various tissues. Animals were injected with 5 mCi ^{3}H-deoxyglucose/kg body weight and tissues were obtained at times indicated. Deoxyglucose-6-phosphate was separated by ion exchange chromatography (see 'Methods'). Values are means of four to six determinations each

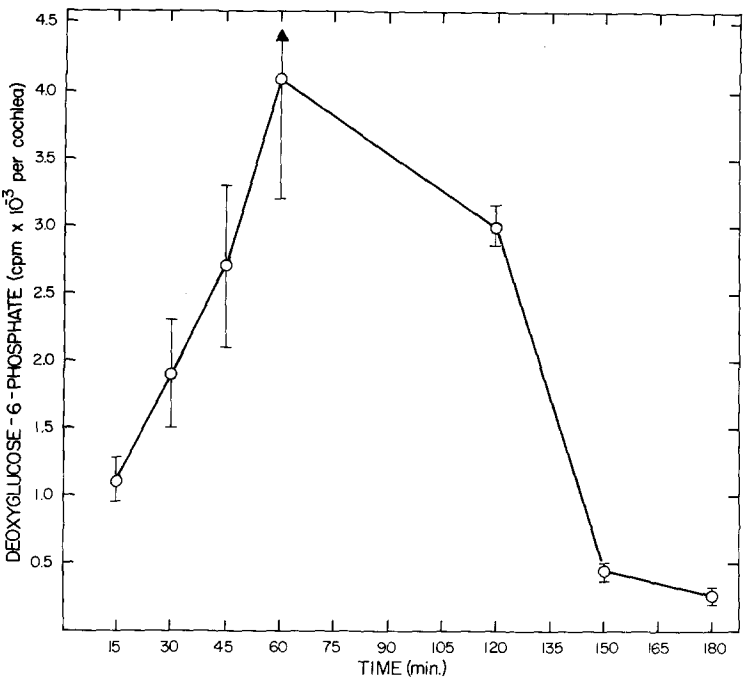

Fig. 2. Kinetics of deoxyglucose-6-phosphate in the cochlea. Experiments were performed as described for Fig. 1. Values are means ± SD of four to six experiments each

Table 1. Effect of noise on deoxyglucose uptake. Animals received 5 mCi ^{3}H-deoxyglucose/kg body weight and were killed after 60 min. Deoxyglucose uptake is expressed as radioactivity per µg tissue protein and is corrected for the integrated serum levels of the tracer (0–60 min). Numbers are means ± SD of four control experiments and three noise exposures (two cochleae per experiment). The results are representative of a series of similar experiments on 11 noise-exposed animals and 12 controls

Tissue	30 dB	100 dB
	Deoxyglucose uptake	
Organ of Corti	154 ± 52	82 ± 18[a]
Stria vascularis/spiral ligament	186 ± 89	78 ± 41[a]

[a] $p < 0.04$

In animals exposed to noise (100 dB, free field, white noise) uptake of deoxyglucose was decreased by about 50% in both tissue preparations at 60 min (Table 1). A 75% decrease was observed in a small group of animals after 120 min of exposure. The significance of these differences was $p < 0.02$ for the two exposure times (one-way ANOVA) and $p < 0.04$ for the 60 min exposure alone (Student's *t*-test). There was no change in the ratio of deoxyglucose-6-phosphate to deoxyglucose in the noise-exposed group.

Discussion

The inner ear utilizes glucose at a lower rate than the brain as seen from the trapping of deoxyglucose. This appears in good agreement with previous studies of the levels of glycolytic enzymes in the inner ear (Thalmann et al. 1970). The steady-state levels of deoxyglucose-6-phosphate as well as the half-life of this compound are significantly lower than in brain. This suggests that glucose-6-phosphatase is present in the cochlea and that trapping in this tissue is rather inefficient. Our techniques of arresting enzymatic activities by microwaving and dissecting in the dry state should help to eliminate artifacts due to dephosphorylation of deoxyglucose-6-phosphate and diffusion of deoxyglucose out of the tissues. It seems that phosphatase activity needs to be carefully considered in radioautographic analyses of deoxyglucose trapping in inner ear tissues.

The salient finding of this study is the dramatic decrease of deoxyglucose uptake in noise exposed animals which is in direct contrast to the metabolic reaction of the CNS to stimuli (Sokoloff 1977). When comparing different groups of animals, as in our study, it is important to note that the amount of radioactive deoxyglucose in a tissue is not only determined by metabolism in the tissues themselves. It may be influenced by systemic factors such as serum glucose levels or the kinetics of deoxyglucose in serum. Our preliminary measurements indicate, however, that there are no differences in these parameters between the two groups of animals. The specificity of the response of the cochlea was further underscored by the finding that tissues such as heart, liver, and kidney do not significantly alter their deoxyglucose uptake in response to noise.

It should be noted our data can not be taken to mean that the hair cells proper decrease their energy metabolism when processing sound information. The dissected preparation termed "organ of Corti" contains supporting cells in addition to the hair cells and is too crude to permit such conclusions. The interpretation of the rather general decrease of cochlear metabolism induced by sound remains speculative and further experiments are necessary. Vasoconstriction of cochlear blood vessels and consequently lowered blood flow and oxygen tension may be contributing factors (Hawkins 1971). Information about hair cell responses will require radioautography at the electron-microscopic level (Sans et al. 1980).

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Note Added in Proof

Experiments currently in progress suggest that deoxyglucose activity at 30 dB already reflects a stimulated state of metabolism. The uptake at sound levels of approximately 10 dB is significantly lower than the uptake at either 30 or 100 dB.