

GLUCOSE UTILIZATION IN THE AUDITORY SYSTEM: COCHLEAR DYSFUNCTIONS AND SPECIES DIFFERENCES

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Abstract—1. Deoxyglucose uptake in peripheral auditory tissues and inferior colliculus of the young CBA or C57BL/6J mouse increases with intensity of an acoustic stimulus from 25 to 85 dBA and decreases from 85 to 115 dBA.

2. In 36-month-old CBA and 9-month-old C57BL with sensorineural hearing loss the metabolic response to stimulation is attenuated 50% or more.

3. Metabolic responses to stimuli are qualitatively and quantitatively different in the gerbil and in the guinea-pig.

4. It is concluded that cochlear dysfunctions are characterized by abnormal evoked metabolism; and that the stimulus-response pattern varies with species.

INTRODUCTION

Although our present understanding of metabolism and blood circulation in the human inner ear is rudimentary, disorders of both have been implicated as an underlying cause for several inner ear dysfunctions. Anatomical studies have shown pathological changes in cochlear blood vessels in patients with severe presbycusis (Johnsson and Hawkins, 1972); sudden hearing loss and fluctuating hearing loss have been suggested to have a similar etiology (Mazzoni, 1969, Maass, 1981). While direct evidence is scant and controversial, animal experiments show that alterations in cochlear blood flow can directly affect local metabolism and auditory thresholds. For instance, by experimentally limiting the blood supply to the cochlea, Thalmann *et al.* (1972) induced a concurrent decrease in cochlear potentials and energy metabolites in cochlear tissues. Little is known, however, how blood flow or metabolism is affected in animal models of certain cochlear disorders.

With the development of the 2-deoxyglucose technique for both animals (Sokoloff *et al.*, 1977) and humans (Reivich *et al.*, 1977), it has been firmly established that physiological stimuli evoke increased energy metabolism in discrete regions of the central nervous system (Phelps *et al.*, 1979; Alavi *et al.*, 1981; Sokoloff, 1981). We have recently reported alterations of deoxyglucose uptake in the cochlea of the normal mouse during acoustic stimulation (Canlon and Schacht, 1983). The aim of the present study was to determine whether the response of glucose metabolism to auditory stimulation is altered in cochlear dysfunctions. We chose the aged CBA mouse and the C57BL/6J mouse, a mutant with genetically determined progressive hearing loss, as animal models for sensory hearing loss. Furthermore, since species differences are known to exist in anatomy and in electrophysiological responses of the auditory system we tested the metabolic response to acoustic stimu-

lation in the gerbil and the guinea-pig. This knowledge is essential before generalizations about auditory metabolism can be made.

MATERIALS AND METHODS

Animals

Four-month-old CBA and C57BL/6J mice (*Mus musculus*, 18–22 g) as well as male CBA mice, 36 months of age (24–28 g) were obtained from the Jackson Laboratory, Bar Harbor, MA; Mongolian gerbils (*Meriones unguiculatus*, 50–60 g) from Tumblebrook Farms, West Brookfield, MA, and albino guinea pigs (*Cavia cobaya*, 200–250 g) from Charles River, Wilmington, MA. All animals had been maintained on Purina Laboratory Chow and water *ad lib.* in a pathogen-free area with exposure to ambient sound. The aged CBA mice were provided through the National Institute on Aging.

Deoxyglucose uptake

Conscious mice and gerbils received a single injection of 5 mCi [³H]deoxyglucose/kg body weight (Pathfinder Lab., St. Louis, MO; specific activity, 40 mCi/mmol) into either the tail vein (mice) or by intracardiac puncture (gerbils). Immediately after the injection animals were placed in a sound shielded exposure box for 60 min. The animals were then killed by decapitation and blood samples taken. Inner ear fluids were absorbed with cotton swabs and the brain and auditory bulla were exposed to microwave irradiation for 15 sec to arrest enzymatic activities. The cochlear tissues were dissected, homogenized, and analyzed for protein (Lowry *et al.*, 1952), and radioactivity. Deoxyglucose-6-phosphate was separated from deoxyglucose by ion exchange chromatography (Hassell *et al.*, 1975). A more complete description of the technique is given elsewhere (Canlon and Schacht, 1983).

Cochlear perfusions

Perilymphatic perfusions were carried out in guinea pigs as previously described (Takada and Schacht, 1982). In brief, glass capillaries were implanted into scala tympani and scala vestibuli of the basal turn of the cochlea after a conventional ventral surgical approach. Artificial perilymph

containing $10 \mu\text{Ci}$ [^3H]deoxyglucose/ml was perfused for 60 min at a rate of approximately $30 \mu\text{l}/\text{min}$. The cochlear microphonic potential was periodically monitored via a recording electrode in scala tympani. At the end of the experiment the animal was decapitated, the cochlea removed and tissues were processed as above.

Sound exposure

Auditory stimuli were presented to mice and gerbils as free-field broadband noise (100 Hz–45 kHz) at intensities of up to 115 dBA. The spectrum was flat to 20 kHz and attenuated approximately 20 dB between 20 and 45 kHz. Noise levels were measured with a half inch condenser microphone on the dBA scale. The exposure box ($40 \times 40 \times 40 \text{ cm}$) was placed inside a soundproof booth in order to obtain the lowest noise level (25 dBA). For the guinea pigs broadband noise (50 Hz–10 kHz) at intensities up to 100 dB was delivered by a calibrated earphone attached to a speculum placed directly before the tympanic membrane. Attenuation at 3 kHz was approximately 10 dB, at 5 kHz approximately 25 dB. To attain the lowest possible noise level in an ambient environment, the incudostapedial joint was separated.

Calculations

For mice and gerbils, deoxyglucose uptake was calculated from the radioactivity in the tissue per μg tissue protein and corrected for the serum levels of [^3H]deoxyglucose (Canlon and Schacht, 1983). For comparisons, activity is expressed relative to the "basal" activity at the lowest attainable noise level. For the guinea-pig, uptake was calculated from tissue radioactivity per μg tissue protein and corrected for the concentration of [^3H]deoxyglucose in the perfusate.

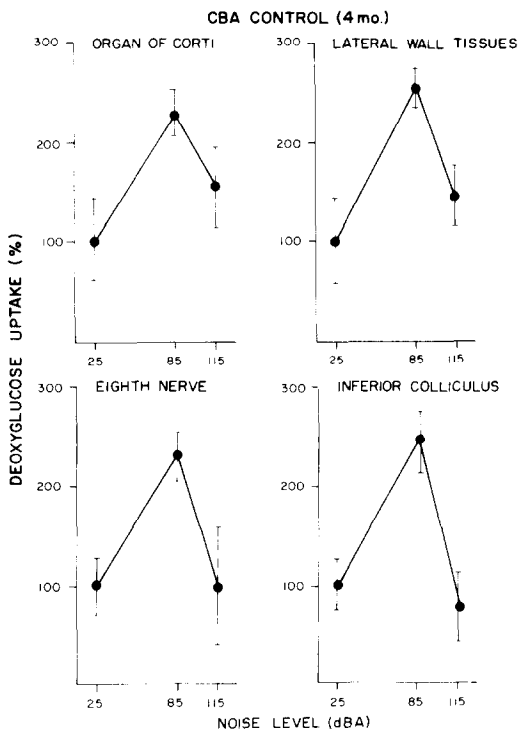


Fig. 1. Response of deoxyglucose uptake to noise exposure in young CBA mice. Animals (4 months old) received a pulse of 5 mCi [^3H]deoxyglucose/kg body weight and were killed after 60 min of exposure to the noise levels indicated. Deoxyglucose uptake was determined as described in the "Materials and Methods" section. Basal rates (at 25 dBA, i.e., 100%) were organ of Corti, 5.6 ± 2.2 ($N = 8$); lateral wall, 6.5 ± 3.1 (8); VIIIth nerve, 6.7 ± 1.9 (7) and inferior colliculus 7.9 ± 2.2 (14) (Canlon and Schacht, 1983).

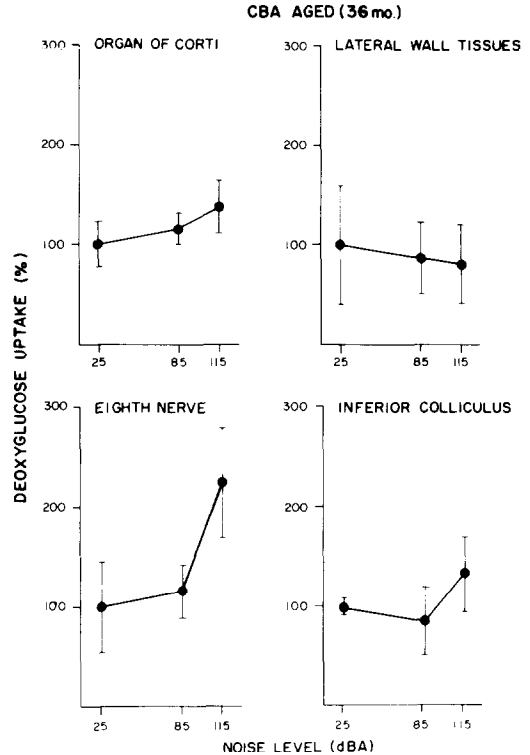


Fig. 2. Response of deoxyglucose uptake to noise exposure in aged CBA mice. Animals (36 months old) were treated as in Fig. 1 and the "Materials and Methods" section. Basal rates (at 25 dBA, i.e., 100%) were organ of Corti, 3.5 ± 0.8 ; lateral wall, 7.1 ± 4.4 ; VIIIth nerve, 3.6 ± 1.8 ; inferior colliculus 14.2 ± 1.1 . Values are means \pm SD from four animals each.

RESULTS

The effect of auditory stimulation on cochlear metabolism in the 36-month-old CBA mouse was compared to the 4-month-old CBA mouse. For the latter, we previously reported a biphasic response to auditory stimulation in the neural auditory structures as well as the lateral wall tissues (Canlon and Schacht, 1983). Cochlear metabolism is represented (Fig. 1) at the critical intensities of 25–85–115 dBA, which correspond to the basal activity, the maximum stimulated response and the decrease from the maximum, respectively. In the aged CBA mouse (Fig. 2), the metabolic response shows a different pattern: at 85 dBA it is approximately 50% lower and at higher intensities there is a suggestion of further increased metabolism in the eighth nerve and inferior colliculus, and to a lesser extent, the organ of Corti. Even at the high intensity (115 dBA) glucose uptake is stimulated less than at 85 dBA in the young, and there is no evidence of a biphasic response.

The C57BL/6J mouse at 3 months of age demonstrated a similar biphasic response to noise stimulation as the young CBA (Fig. 3). The 9-month-old C57BL/6J mouse still displayed a biphasic response to noise stimulation yet the maximum was reduced by approximately 50% (Fig. 4).

The auditory tissues of the gerbil show a linear response to noise and a lesser stimulation; only at

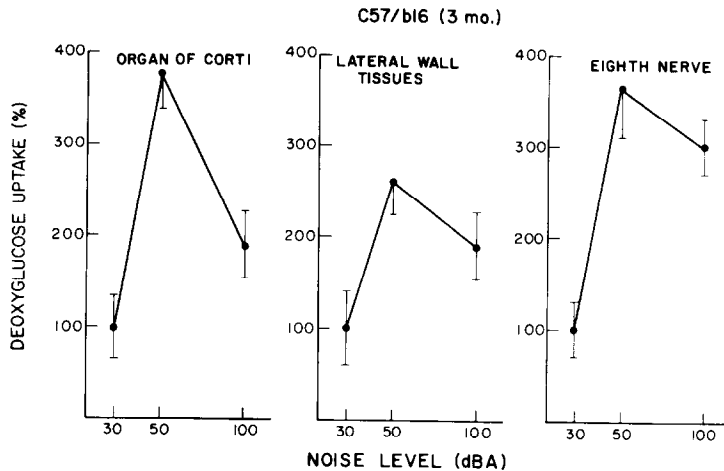


Fig. 3. Response of deoxyglucose uptake to noise exposure in young C57BL/6J mice. Animals (3 months old) were treated as in Fig. 1 and "Materials and Methods" section. Basal rates (at 30 dBA, i.e. 100%); organ of Corti, 2.1 ± 0.7 ; lateral wall, 3.2 ± 1.3 ; VIIIth nerve 2.6 ± 0.8 . Values are means \pm SD from three animals each.

115 dBA does the deoxyglucose uptake reach a relative magnitude comparable to that in the mouse at 85 dBA (Fig. 5). A biphasic response could not be observed at the intensities tested.

Deoxyglucose uptake in the guinea pig cochlea was studied by continuous perilymphatic perfusion. The

time course for incorporation of [3 H]deoxyglucose was linear up to 2 hr, the longest time investigated. After 60 min, steady state levels of deoxyglucose-6-phosphate were attained. There was no significant increase in metabolism in response to noise of 25–100 dB (Fig. 6).

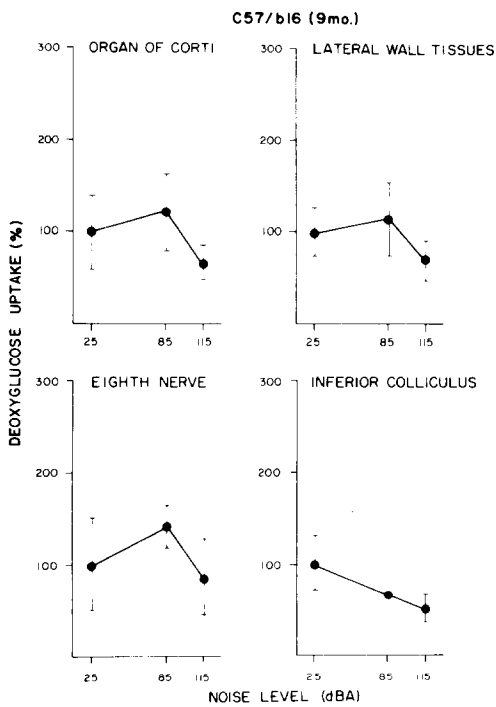


Fig. 4. Response of deoxyglucose uptake to noise exposure in 9-month-old C57BL/6J mice. Animals were treated as in Fig. 1 and "Materials and Methods" section. Basal rates (25 dBA, i.e. 100%) were organ of Corti 4.7 ± 2.1 ; lateral wall, 6.9 ± 1.8 ; VIIIth nerve 5.7 ± 3.4 ; inferior colliculus 18.2 ± 5.8 . Values are means \pm SD from three animals each.

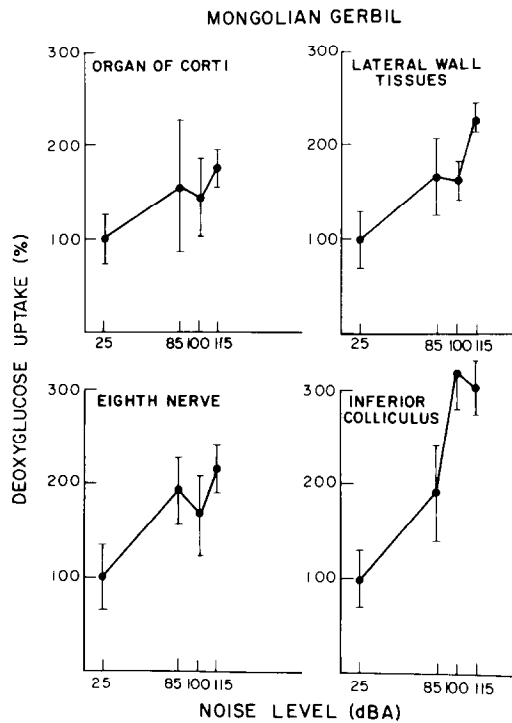


Fig. 5. Response of deoxyglucose uptake to noise in Mongolian gerbils. Animals (3–4 months old) were treated as in Fig. 1 and "Materials and Methods" section. Basal rates (25 dBA, i.e. 100%) were organ of Corti 5.5 ± 1.5 ; lateral wall 5.0 ± 1.3 ; VIIIth nerve 4.2 ± 1.6 ; inferior colliculus 5.2 ± 1.8 . Values are means \pm SD of 3–4 animals each.

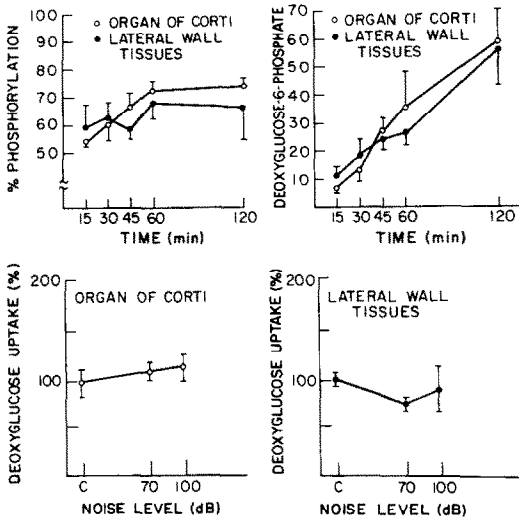


Fig. 6. Deoxyglucose uptake in the guinea-pig cochlea. Animals received perilymphatic perfusions with deoxyglucose ($10 \mu\text{Ci/ml}$) as described in "Materials and Methods" section. Upper panels: time course of phosphorylation and of accumulation of deoxyglucose-6-phosphate. Lower panels: effect of noise on deoxyglucose uptake. "C" on x-axis indicates lowest noise level achieved by separating the incudostapedial joint. Values are means \pm SD of 4–6 animals each.

DISCUSSION

Cochlear glucose utilization in response to acoustic stimulation is significantly changed in the aged CBA and the elder C57BL/6J mouse. It should be emphasized that in this study we compare the magnitude of the metabolic response and the stimulus-response pattern only. Other comparisons, e.g. of metabolic rates between species, would require knowledge of the "lumped constant" but its determination for individual inner ear tissues is technically not feasible (Canlon and Schacht, 1983). Even comparisons of metabolic rates between young and old animals of the same strain may be influenced by a change in the lumped constant with age (Takei *et al.*, 1983).

The reduced metabolic response in aged animals of both strains appears to correlate with reported morphological and electrophysiological findings of degenerative changes of the peripheral auditory system. Morphological studies have established hair cell and nerve fiber pathology with increasing age in humans (Johnsson and Hawkins, 1972), rats (Keithley and Feldman, 1982) and C57BL/6J mutants (Mikaelian, 1979), as well as in the CBA mice used in this study (Hawkins, personal communication). In addition, the electrical response to sound is depressed with age as shown in guinea pigs (Pestalozza *et al.*, 1957), rats (Crowley *et al.*, 1972), C57BL/6J mutants (Mikaelian, 1979), and in the aged CBA mice (Nuttall, personal communication). It is thus conceivable that the loss of sensory cells is the cause of the depressed metabolic response to acoustic stimulation. It remains open, however, whether a metabolic deficiency may have preceded and caused physiological dysfunction and anatomical pathology.

In contrast to the depression of stimulated metabolism, the values measured at the lowest attainable

noise level did not change in any consistent pattern with age in the CBA and C57BL/6J mice. Even considering the possibility of altered lumped constants with age it is evident that the evoked response of auditory structures is more clearly affected than the apparent basal metabolism. This appears essentially in agreement with a report that central auditory pathways showed reduced glucose utilization in aged rats at ambient (i.e. stimulatory) noise levels (Smith *et al.*, 1980).

The second aim of this study was to determine whether auditory metabolism is as varied among species as are the anatomy and the electrophysiological responses: different species have unique characteristic frequencies, threshold curves and frequency ranges. Our results show that the pattern of the metabolic response of the mouse is not a general feature of auditory systems. While all peripheral tissues, including those of the cochlear lateral wall, are responsive to stimulation in the gerbil, the pattern differs from the mouse in two aspects. First, there is a linear increase with elevated noise levels without indication of a biphasic response for the intensities investigated. Secondly, at moderate sound intensities, the gerbil shows approximately half the metabolic stimulation of the mouse. These findings apparently agree with those of Ryan *et al.* (1982), who by autoradiographic analysis found little increases of deoxyglucose uptake with noise stimulation in the gerbil.

The guinea-pig was chosen for comparisons because of its well characterized auditory system. Moreover, cochlear perfusions provide a convenient means of direct and continuous deoxyglucose application while allowing manipulation and control of cochlear function. Perilymphatic glucose has been demonstrated to be a substrate for maintaining cochlear potentials (Kambayashi *et al.*, 1982), but we did not observe a stimulus related increase of deoxyglucose uptake into auditory tissues. Since the route of administration bypasses the blood circulation this could indicate that blood flow is mediating the response to acoustic stimulation in analogy to coupling of blood flow and metabolism in brain (Reivich, 1974). However, it should be kept in mind that the perfusion requires the animal remaining anesthetized during the experiment, and this may affect metabolism (Sokoloff *et al.*, 1977).

In conclusion, the magnitude of the metabolic stimulation of auditory tissues and the pattern of the dose-response relationship varies with species and possibly the route of precursor administration. However, our results indicate that the cochlear dysfunctions investigated in these animal models are characterized by abnormal evoked metabolism. Assessments of auditory metabolism in other well characterized disorders should shed more light on the relationship between cochlear metabolism and pathology but such studies must utilize defined sound stimulus levels and generalizations across species should be made with great caution.

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REFERENCES

- Alavi A., Reivich M., Greenberg J., Hand P., Rosenquist A., Rintelmann W., Christman D., Fowler J., Goldman A., MacGregor R. and Wolf A. (1981) Mapping of functional activity in brain with [¹⁸F]-Fluoro-deoxyglucose. *Sem. Nucl. Med.* **XI**, 24-31.
- Canlon B. and Schacht J. (1983) Acoustic stimulation alters deoxyglucose uptake in the mouse cochlea and inferior colliculus. *Hearing Res.* **10**, 217-226.
- Crowley D. E., Swain R. E. and Schramm V. L. (1972) Analysis of age-related changes in electrical responses from the inner ear of rats. *Ann. Otol.* **81**, 739-749.
- Hassell J. A., Colby C. and Romano A. (1975) The effect of serum on the transport and phosphorylation of 2-deoxyglucose by untransformed and transformed mouse 3T3 cells. *J. Cell Physiol.* **86**, 37-45.
- Johnsson L.-G. and Hawkins J. E., Jr. (1972) Sensory and neural degeneration with aging, as seen in microdissections of the human inner ear. *Ann. Otol.* **81**, 179-193.
- Kambayashi J., Kobayashi T., Marcus N. Y., DeMott J. E., Thalmann I. and Thalmann R. (1982) Minimal concentrations of metabolic substrates capable of supporting cochlear potentials. *Hearing Res.* **7**, 105-114.
- Keithley E. M. and Feldman M. L. (1982) Hair cell counts in an age-graded series of rat cochleas. *Hearing Res.* **8**, 249-262.
- Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265-275.
- Maass B. (1981) Physiological and pathological aspects of inner ear circulation. *Proc XIIIth ORL World Congr.* (Edited by Surjan L. and Bodo Gy.), pp. 607-609. Hungarian Academy of Science, Budapest.
- Massoni A. (1969) Internal auditory canal arterial relations at the porus acusticus. *Ann. Otol.* **78**, 797-814.
- Mikaelian D. O. (1979) Development and degeneration of hearing in the C57/b16 mouse: relation of electrophysiologic responses from the round window and cochlear nucleus to cochlear anatomy and behavioral responses. *Laryngoscope* **89**, 1-15.
- Pestalozza G., Davis H., Eldredge D. H., Covell W. P. and Rogers J. B. (1957) Decreased bio-electric potentials in the ears of senile guinea pigs. *Laryngoscope* **67**, 1113-1122.
- Phelps M. E., Huang S. C., Hoffman E. F., Selin M. S., Sokoloff L. and Kuhl D. E. (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxyglucose: Validation of method. *Ann. Neurol.* **6**, 371-388.
- Reivich M. (1974) Blood flow metabolism couple in brain. *Res. Publ. Ass. Res. nerv. ment. Dis.* **53**, 125-140.
- Reivich M., Kuhl D., Wolf A., Greenberg J., Phelps N., Ido T., Casella V., Fowler J., Gallager B., Hoffman E., Alavi A. and Sokoloff L. (1977) Measurement of local cerebral glucose metabolism in man with ¹⁸F-2-fluoro-2-deoxy-D-glucose. *Acta Neurol. scand. (Suppl.)* **56(64)**, 190-191.
- Ryan A. F., Goodwin P., 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.
- Smith C. B., Gooch C., Rapoport S. I. and Sokoloff L. (1980) Effects of aging on local rates of cerebral glucose utilization in the rat. *Brain* **103**, 351-365.
- Sokoloff L. (1981) The deoxyglucose method for the measurement of local glucose utilization and the mapping of local functional activity in the central nervous system. *Int. Rev. Neurobiol.* **22**, 287-333.
- Sokoloff L., Reivich R. M., Kennedy C., Des Rosiers M. H., Patlack C. S., Pettigrew K. D., Sakurada O. and Shinohara M. (1977) The ¹⁴C-deoxyglucose method for measurement of local cerebral glucose utilization. *J. Neurochem.* **28**, 897-916.
- Takada A. and Schacht J. (1982) Calcium antagonism and reversibility of gentamicin-induced loss of cochlear microphonics in the guinea pig. *Hearing Res.* **8**, 179-186.
- Takei H., Fredericks W. R., London E. D. and Rapoport S. I. (1983) Cerebral blood flow and oxidative metabolism in conscious fischer-344 rats of different ages. *J. Neurochem.* **40**, 801-805.
- Thalmann R., Miyoshi T. and Thalmann I. (1972) The influence of ischemia upon the energy reserves of inner ear tissues. *Laryngoscope* **82**, 2249-2272.