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Effect of aging on *myo*-inositol and phosphoinositide metabolism in the cochlear and vestibular sensory epithelia of the rat

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Abstract

Neurotransmission and transmembrane signaling are among the cellular mechanisms affected in the aging nervous system. In the inner ear, the phosphoinositide second messenger cascade is of particular interest as a target of the aging process. In both the cochlear (CSE) and vestibular sensory epithelia (VSE), the hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) to the second messenger inositol 1,4,5-trisphosphate ($InsP_3$) is coupled to muscarinic cholinergic and P_{2y} purinergic receptors and may be linked to calcium homeostasis. The present study compared the turnover of phosphoinositides (PtdInsPs), receptor-mediated release of inositol phosphates (InsPs), and concentrations of endogenous *myo*-inositol in the CSE and VSE of young (3 months) and aged (24 months) Fischer-344 rats. In the aged rat, there was a significant increase in [^{3}H]inositol incorporation (per mass of protein) into PtdInsPs plus InsPs in both sensory epithelia while the protein content remained unchanged. In contrast, no age-dependent differences were found when pre-labeled [^{3}H]PtdInsPs were 'chased' with non-radiolabeled *myo*-inositol indicating that the turnover of these lipids was unaffected. The cholinergic receptor agonist carbamylcholine and the P_2 purinergic receptor agonist adenosine 5′-*O*-(3-thiotriphosphate) stimulated the release of [^{3}H]InsPs two- to six-fold in both organs. This agonist-stimulated release of [^{3}H]InsPs (per mass of protein) was significantly higher in aged animals. However, when the same stimulation was expressed as per cent of control values, there was no age-dependent difference. Finally, the concentration of endogenous *myo*-inositol decreased by 44% in the aged CSE and by 24% in the aged VSE. In contrast, levels of added *myo*-[^{3}H]inositol were higher in aged tissues. These results suggest that the increased labeling of PtdInsPs and InsPs in the aged CSE and VSE is a consequence of the increased specific radioactivity of the *myo*-[^{3}H]inositol precursor pool. The activity of the phosphoinositide second messenger pathway thus appears unchanged. However, a decreased *myo*-inositol content may contribute to age-dependent pathology in these tissues. *myo*-Inositol is an organic osmolyte and volume regulator. Changes in osmotic pressure or turgor of hair cells could alter micromechanical coupling on the basilar membrane and vestibular epithelium causing pathophysiological changes in sensory transduction.

Key words: Aging; Inositol phosphates; Phosphoinositides; *Myo*-inositol; Second messenger; Cochlea; Vestibule; Rat, Fischer-344

1. Introduction

Age-related decreases in hearing sensitivity and increased incidence of equilibrium dysfunction are well documented in the human (Schuknecht, 1974; Oosterveld, 1983) and in experimental animals (Willott, 1991, for review). In both the cochlea and vestibule, one prominent morphological change during aging is the gradual loss of hair cells. In the cochlea, hair cells are characteristically lost at one or both extremes of the organ of Corti, with a higher susceptibility of the outer hair cells in most species. However, physiological determinations of sensory deficits frequently do not correspond to hair cell pathology, suggesting that more subtle changes in cell function may underlie some of the age-related dysfunctions.

Neurotransmitter mechanisms and intracellular homeostasis and regulation are among the metabolic processes affected in the aging nervous system (Gibson and Peterson, 1987; Barritt, 1987; for review). Signal transduction may be a specific target and in particular, the inositol 1,4,5-trisphosphate ($InsP_3$) second messenger system. $InsP_3$ is produced by the receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) in response to neurotransmitters and hor-

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mones (Henzi and MacDermott, 1992; Berridge, 1993; Irvine, 1993; for review). It serves to elevate intracellular calcium which, in turn, plays a central role in cell physiology by controlling a diversity of biochemical events ranging from metabolic regulation to cell death. Aging affects the $InsP_3$ second messenger system in various organs, including brain (Crews et al., 1986; Nalepa et al., 1989; Burnett et al., 1990a,b; Joseph et al., 1990; Mundy et al., 1991; Ohnuki and Nomura, 1991; Kurian et al., 1992; Undie and Friedman, 1992), pineal gland (Tandon et al., 1991; Laitinen et al., 1992), pituitary gland (Bonnetti et al., 1987), liver (Borst and Scarpace, 1990), heart (Miyamoto and Ohshika, 1989; Moscona-Amir et al., 1989), parotid gland (Ishikawa et al., 1988), tracheal smooth muscle (Willis-Karp, 1991), granulocyte (Fulop et al., 1989) and platelets (Bastyr et al., 1990).

In addition to being a precursor of the phosphoinositides, *myo*-inositol is an important factor in cell growth and maintenance. Nutritional deficits or decreases in its tissue content have been implicated in pathological processes leading to inadequate growth and even cell death (see review by Wells, 1989). At the cellular level, *myo*-inositol acts as a major osmoregulator in the kidney (Garcia-Perez and Burg, 1990) and in other tissues including brain (Paredes et al., 1992). In the aged human brain, *myo*-inositol is decreased to half the level in young brain (Stokes et al., 1983).

In the cochlear (CSE) and vestibular sensory epithelia (VSE) of guinea pig and rat the $InsP_3$ second messenger system is coupled to muscarinic and purinergic P_{2y} receptors (Guiramand et al., 1990; Niedzielski and Schacht, 1991, 1992; Niedzielski et al., 1992; Ogawa and Schacht, 1993a) and may thus be linked to neurotransmission and modulation. Nothing is known about the possible role of *myo*-inositol in cochlear or vestibular osmoregulation. In this study, we compared the activity of the phosphoinositide second messenger system and the concentrations of *myo*-inositol in the CSE and VSE of 3-month-old and 24-month-old Fischer-344 rats. Around 24 months of age, rats show electrophysiological changes in the auditory periphery such as elevation of thresholds to clicks and tone pips (Cooper et al., 1990; Simpson et al., 1985). Some of these results have been presented in a preliminary report (Ogawa and Schacht, 1993b).

2. Materials and methods

2.1. Materials

Fischer-344 rats (3 months old, male; Charles River Lab., Kingston, NY; and 24 months old, male, National Institute of Aging Colonies, obtained through Harlan-Sprague-Dawley, Indianapolis, IN) were used in this study. *myo*-[^{3}H]Inositol (specific activity 82 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Hanks' balanced salt solution (HBSS) from Gibco BRL Life Technologies (Gaithersburg, MD), and high performance thin layer chromatography (HPTLC) plates (Silica gel 60) from Merck (Cherry Hill, NJ). Other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Tissue preparation

Rats were decapitated, the otic capsules quickly removed from the temporal bone and placed in incubation buffer (see below) at room temperature. The otic capsule was opened and the sensory epithelia were removed by microdissection and kept in the incubation buffer. The tissue defined here as 'cochlear sensory epithelium' (CSE) consisted of inner and outer hair cells, supporting cells, basilar membrane and the lateral part of the spiral limbus; the 'vestibular sensory epithelium' (VSE) contained macula utriculi, macula sacculi and cristae ampullares from the semicircular canals. The incubation buffer was HBSS (137.9 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 0.3 mM KH_2PO_4, 5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 5.6 mM D-glucose) with 5 mM sodium *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). It was gassed with 95% O_2/5% CO_2 for 30 min, its pH titrated to 7.4 ± 0.01 with NaOH and then its osmolality adjusted to 300 ± 2 mOsm with NaCl.

2.3. Labeling of inositol phospholipids and release of inositol phosphates

Methods for radioactive labeling of phosphoinositides and the separation of inositol phosphates (InsPs) were essentially as previously reported (Niedzielski and Schacht, 1991; Ogawa and Schacht, 1993a). Tissues were placed in 50 μl of incubation buffer with 1 mM cytidine and 16 μCi *myo*-[^{3}H]inositol for 2 h at 37°C. *myo*-[^{3}H]Inositol was removed by two washes (0.5 ml each) with buffer free of radioactivity. Incubation continued for 10 min in 90 μl of buffer with 10 mM LiCl. Finally, agonists were added in 10 μl of buffer to yield concentrations of 1 mM carbamylcholine (CCh) or 200 μM adenosine 5'-*O*-(3-thiotriphosphate) (ATP-γ-S) and the incubation was continued for 30 min. The duration of the incubation assured optimal labeling and viability of the tissues (Niedzielski and Schacht, 1991; Niedzielski et al., 1992). Phosphoinositide hydrolysis was terminated with 300 μl of chloroform/methanol (1:2 by vol). One hundred μl of phytic acid hydrolysate (prepared by heating 4 mg phytic acid in 1 ml of 30 mM acetic acid/65 mM sodium acetate for 48 h at 95°C, quenching with 20 μl of 1N NaOH and diluting 1:100 with H_2O) and 200 μl of bovine brain extract in

chloroform (0.2 mg of Sigma type IV/ml) were added as carriers to reduce loss of labeled InsPs and lipids during their isolation. The aqueous and chloroform phases were separated by centrifugation. One hundred μl of the chloroform phase were dried down before radioactivity in phosphoinositide lipids was determined by liquid scintillation counting.

InsPs in the aqueous phase were separated from *myo*-inositol after Berridge et al. (1983) and Dean and Beaven (1989) with minor modifications. Two hundred μl of sample were diluted to 1.5 ml with distilled water and passed over Dowex-1 formate (cross-linkage 8%, mesh size 200–400; column, 25 × 5 mm). The column was first washed with 8 ml of 5 mM *myo*-inositol. Individual inositol phosphates were separated by sequential elution with 5 ml of 5 mM sodium tetraborate in 60 mM sodium formate (glycerophosphoinositol, GPI), 0.2 M ammonium formate in 0.1 mM formic acid (inositol monophosphate, InsP), 0.4 M ammonium formate in 0.1 mM formic acid (inositol bisphosphate, $InsP_2$), 1 M ammonium formate in 0.1 mM formic acid (inositol trisphosphate, $InsP_3$). When total combined InsPs were measured, the sequential steps were replaced by a single elution with 5 ml of 1 M ammonium formate in 0.1 M formic acid. Radioactivity of each fraction was determined by liquid scintillation counting. Columns were used for 5 experiments by regenerating with 10 ml of 1 M sodium formate in 0.1 M formic acid and 10 ml of distilled water.

2.4. Hydrolysis of inositol phospholipids

The hydrolysis of phosphoinositides was measured in a 'chase' experiment. Tissues were labeled for 2 h with *myo*-[^{3}H]inositol as described above, washed and further incubated with 1 mM non-radioactive *myo*-inositol at 37°C. After termination of the reaction, the chloroform phase was washed with 500 μl of 2.4 N HCl and an aliquot counted for radioactivity. The remainder of the chloroform phase was dried under N_2 and stored at −20°C until the thin layer chromatographic separation. HPTLC plates were pretreated overnight in methanol/H_2O (2:3, by vol.) containing 0.01 g of potassium oxalate per ml of solvent. Plates were activated by heating at 100°C for 45 min just prior to use. Dried lipids were dissolved in 30 μl of chloroform/methanol (1:2, by vol.) and spotted along with standards of phosphoinositides. The plates were developed for 45 min at 4°C with chloroform/acetic acid/methanol/acetone/H_2O (40:15:13:12:7, by vol.). After drying the plates, phospholipids were visualized by molybdenum blue (0.65% molybdenum oxide in 4.2 M sulfuric acid). Bands corresponding to phosphatidylinositol (PtdIns), phosphatidylinositol 4-monophosphate (PtdInsP) and $PtdInsP_2$ were scraped off and radioactivity was determined by scintillation counting.

2.5. Measurement of myo-inositol

CSE and VSE were homogenized by sonication in 150 μl of HBSS with 5 mM HEPES, pH 7.4, and centrifuged at 3000 × *g* for 5 min. *myo*-Inositol in the supernatant was assayed by high performance liquid chromatography (HPLC), modified from Kargacin et al. (1987). *myo*-Inositol was derivatized by incubating 30 μl of sample with 30 μl each of 10 mM aqueous triethylamine and 10 mM isatoic anhydride in dimethylsulfoxide for 15 min at 60°C. Eighty μl of the mixture were injected onto a nucleotide/nucleoside reversed phase column (250 × 4.6 mm; Alltech Assoc., Deerfield, IL) protected by a guard column with pellicular C18 packing. A linear gradient from 10% methanol/90% aqueous acetic acid, pH 3.4, to 100% methanol was applied in 30 min, followed by a 10-min hold at 100% methanol and a 10-min reverse gradient to the starting conditions. Because of its multiple functional groups, the derivatization resulted in several peaks for *myo*-inositol which were detected with a FD100 filter fluorimeter (GTI/SpectroVision, Concord, MA) using a 330 nm excitation filter and a 400 nm cut-off emission filter. The earliest eluting *myo*-inositol peak was

Table 1
Agonist-stimulated *myo*-[^{3}H]inositol phosphate release

Agonist	CSE		VSE	
	Young	Old	Young	Old
(dpm [^{3}H]InsPs/μg protein)				
Control	4184 ± 590	6826 ± 1596 *	4690 ± 810	6489 ± 1818 *
CCh	8214 ± 1928	14133 ± 2661 **	10021 ± 2386	11752 ± 2619
ATP-γ-S	22089 ± 3793	42270 ± 14787 *	24437 ± 7046	46847 ± 7181 **
(% of control without agonist)				
CCh	196 ± 37	222 ± 86	222 ± 75	195 ± 72
ATP-γ-S	540 ± 141	662 ± 317	594 ± 126	759 ± 172

CSE and VSE were prelabelled with 16 μCi *myo*-[^{3}H]inositol for 2 h and then incubated with 1 mM CCh or 200 μM ATP-γ-S for 30 min. Data are means ± SD of 6 independent experiments. Stimulation in aged tissue differs from that in corresponding young tissue: * $0.01 < P < 0.05$; ** $P < 0.01$ (paired Student's *t*-test).

used for quantification by peak height, calibrated against a *myo*-inositol standard.

2.6. Other procedures

Protein was determined after Bradford (1976) using bovine serum albumin as a standard. Results are reported as means ± SD. Statistical significance of differences between young and aged tissues was tested using two-tailed paired Student's *t*-test.

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3. Results

3.1. Receptor-mediated release of myo-inositol phosphates

In the CSE, basal unstimulated release of [^{3}H]InsPs was 4184 ± 590 dpm/μg protein in young (3 months old) and 6826 ± 1596 dpm/μg protein in aged rats (24 months old). In the VSE, a similar increase with age was seen: total InsPs were 4690 ± 810 dpm/μg protein and 6489 ± 1818 dpm/μg protein in young and aged rats, respectively (Table 1). A trend towards age-dependent increases was also evident in the radioactivity of individual InsPs in both the CSE and VSE (Fig. 1).

CCh stimulated the release of InsPs approximately 2-fold, and ATP-γ-S 5- to 7-fold. Both agonists released more [^{3}H]InsPs (per μg protein) in both the aged CSE and VSE (Table 1). This trend was also seen in individual InsPs (Fig. 1).

3.2. Phospholipid labeling and hydrolysis

Incorporation of *myo*-[^{3}H]inositol (expressed as radioactivity per μg protein) into phosphoinositide lipids, precursors of InsPs, was also increased significantly by age in both the CSE and VSE (Table 2). Radioactivity was primarily found in PtdIns; the polyphosphoinositides, PtdInsP and PtdInsP$_2$, represented about 10% of the total [^{3}H]inositol lipids. There were no age-related changes in the distribution of radioactivity among the phosphoinositides. This pattern was similar between the CSE and VSE and between young and old rats.

The rate of hydrolysis of phosphoinositides was determined by incubating prelabeled lipids with excess non-radioactive *myo*-inositol. Radioactivity in all three phosphoinositides decreased by 40% to 50% during the chase period. There was, however, no significant difference in the rate of hydrolysis with age in any of the lipids in either the CSE and VSE (Fig. 2).

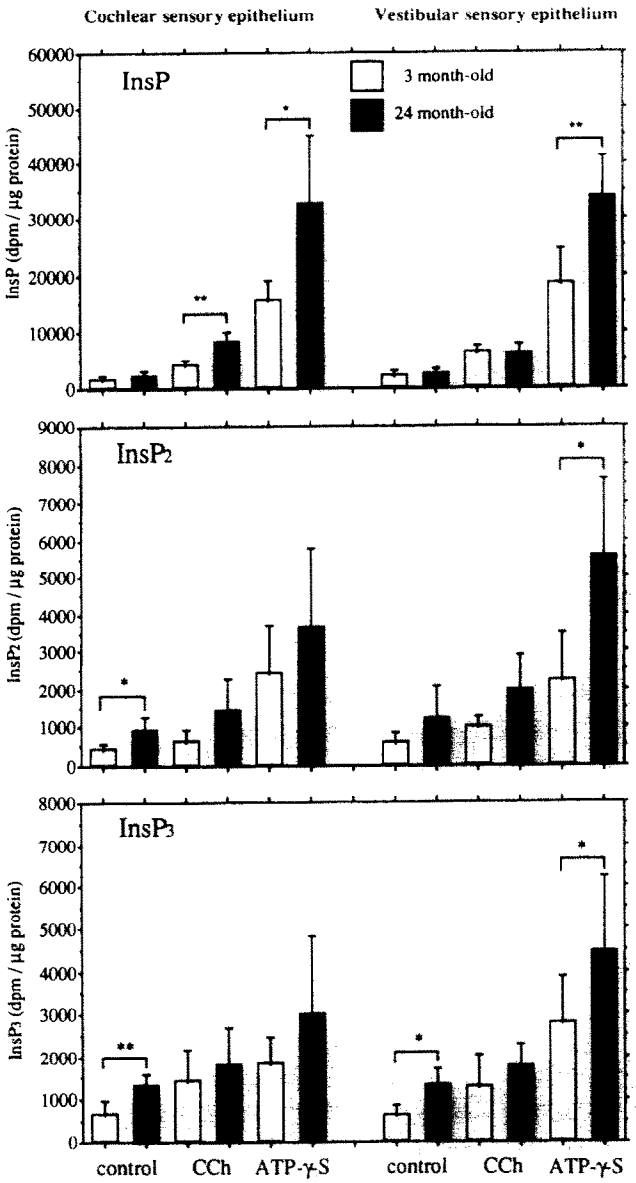

Fig. 1. Effects of aging on agonist-stimulated release of InsP, InsP$_2$ and InsP$_3$. CSE and VSE were prelabelled with 16 μCi *myo*-[^{3}H]inositol for 2 h and then incubated with 1 mM CCh or 200 μM ATP-γ-S for 30 min as described in Materials and methods. The results are expressed as dpm of individual InsPs per μg protein. Each figure is the mean ± SD of 6 independent experiments. Statistical significance of differences between young and aged tissues was determined by paired Student's *t*-test (* $0.01 < P < 0.05$; ** $P < 0.01$).

3.3. Tissue concentration and uptake of myo-inositol

The *myo*-inositol content was 314 ± 96 and 175 ± 28 μmol/μg protein in young and aged CSE, respectively, representing a significant 44% decrease with age. In the VSE, *myo*-inositol content was decreased by 24% in aged rats from 110 ± 27 to 84 ± 8 μmol/μg protein. In contrast to the endogenous *myo*-inositol content, the added *myo*-[^{3}H]inositol reached significantly higher concentrations in the aged tissues (Table 3).

Table 2
myo-[^{3}H]Inositol labeling of phospholipids

Lipid	CSE		VSE	
	Young	Old	Young	Old
(10^{-3} dpm/μg protein)				
PtdInsPs	59.4 ± 4.7	95.3 ± 19.1 *	107.7 ± 5.6	134.0 ± 18.5 *
(% of total lipid radioactivity)				
PtdIns	87.4 ± 2.5	89.1 ± 1.4	90.2 ± 1.4	91.1 ± 1.0
PtdInsP	7.6 ± 1.6	6.6 ± 0.9	6.3 ± 1.1	5.7 ± 0.8
PtdInsP2	3.3 ± 0.6	3.0 ± 1.0	2.3 ± 0.3	2.1 ± 0.3

CSE and VSE were labelled with 16 μCi *myo*-[^{3}H]inositol for 2 h and separated by TLC as described in Materials and methods. Data are means ± SD of 5 independent experiments. Incorporation in aged tissue differs from that in corresponding young tissue: * $0.01 < P < 0.05$ (paired Student's *t*-test).

3.4. Protein content and normalization of data

The protein content of the CSE and VSE remained unchanged with age (10.6 ± 3.1 μg and 10.3 ± 2.3 μg in young and aged CSE; 13.4 ± 4.6 μg and 11.7 ± 3.2 μg in young and aged VSE) providing a basis for normalizing the radiolabeling and comparing results from different experiments as shown above. This normalization reflects the overall incorporation of the added *myo*-[^{3}H]inositol. It does not account for specific relationships between radioactive products and their precursors. This relationship becomes evident when radioactive labeling of PtdInsPs is compared to the *myo*-[^{3}H] inositol pool, and labeling of InsPs to that of PtdInsPs. Similarly, the agonist-stimulated InsPs release can be expressed as percent of the corresponding unstimu-

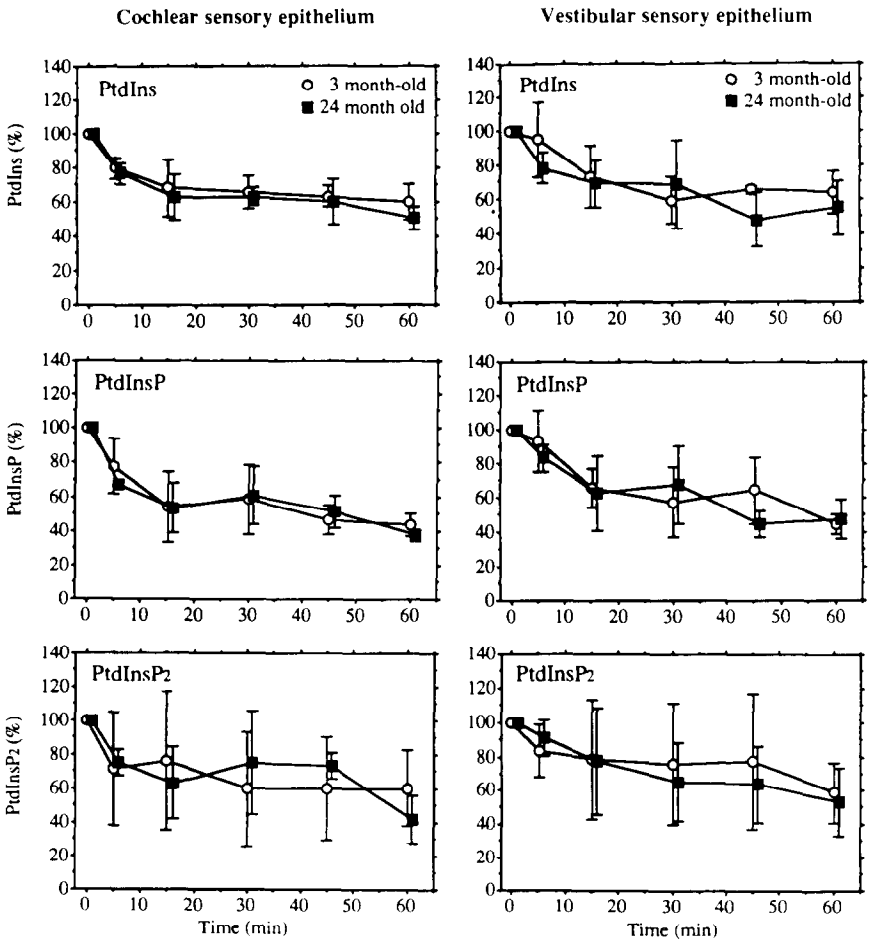

Fig. 2. Hydrolysis of phosphoinositides in young and aged CSE and VSE. Tissues were labeled with *myo*-[^{3}H]inositol and subsequently incubated with 1 mM non-radioactive *myo*-inositol as described in Materials and methods. Results are expressed as % of radioactivity present at 0 min of the 'chase', i.e. after the prelabeling period. Each bar is the mean ± SD of 5 independent experiments.

Table 3
Tissue contents of endogenous *myo*-inositol and of *myo*-[^{3}H]inositol

	CSE		VSE	
	Young	Old	Young	Old
myo-Inositol (μmol/μg protein)	314±96	175±28 *	110±27	84± 8 *
myo-[^{3}H]inositol (10^{-3} dpm/μg protein)	165±40	213±43 **	160±44	229±50 **

myo-Inositol content was measured in individual samples from 4 animals in each category as described in Materials and methods. *myo*-[^{3}H]inositol uptake was determined from 18 experiments as the total radioactivity (InsPs, PtdInsPs and free *myo*-inositol) after incubation with 16 μCi *myo*-[^{3}H]inositol for 2 h as described in Materials and methods. Data are means±SD. Results in aged tissue differ from those in corresponding young tissue: * $0.01 < P < 0.05$; ** $P < 0.01$ (paired Student's *t*-test).

lated control. With these methods of normalization, there was no significant difference with age in lipid labeling (i.e., the pathway from *myo*-inositol to PtdInsPs), in InsPs release (i.e., the pathway from PtdInsPs to InsPs) or in agonist-induced [^{3}H]InsPs release. The last example is shown in Table 1.

4. Discussion

Incorporation of *myo*-[^{3}H]inositol into phosphoinositides and InsPs was significantly enhanced in both the CSE and VSE of 24-month- old rats. Concomitantly, the carbamylcholine- and ATP-stimulated release of [^{3}H]InsPs increased. Since the amount of protein recovered from young and old inner ears remained unchanged, this increase of *myo*-[^{3}H]inositol incorporation is not caused by loss of tissue or by age-related decreases in protein content of these organs.

An accelerated turnover of phosphoinositides or enhanced responsiveness to hormonal stimulation could lead to the observed effects. Alternately, changes in the specific radioactivity of the precursor pool of *myo*-[^{3}H]inositol could be reflected in the radiolabeling of lipids and InsPs. The sum of the experimental evidence suggests that the latter mechanism may account for the results. While the amount of radioactivity is higher in the lipids and InsPs of aged tissues, the *rate* of their formation and their turnover does not seem to be affected. This becomes evident when radioactivity in the products (lipids and InsPs) is compared to radioactivity in the corresponding precursors (*myo*-inositol and PtdInsPs). On this basis, there is no difference between young and aged animals. Supporting this notion is the fact that hydrolysis of PtdInsPs remains unchanged, as measured directly in the 'chase' experiment. Furthermore, agonist-stimulated InsPs release is of the same magnitude (percent stimulation) in aged CSE and VSE. Thus, there appear to be no changes with aging in phosphoinositide turnover and coupling of cholinergic and purinergic receptors to phospholipase C and the formation of InsPs. This finding is also compatible with the absence of differences in the levels of the efferent neurotransmitter acetylcholine between 3-, 12- and 24-month-old rats (Hoffman et al., 1988). The lack of an effect in the inner ear and the fact that both age-dependent increases and decreases of receptor-mediated InsPs release have been observed in other tissues (see references in the introduction) indicate a high degree of complexity of aging effects on the $InsP_3$ second messenger system.

In the synthesis of phosphoinositides, free intracellular *myo*-inositol is incorporated into PtdIns via a reaction with CDP-diglyceride. PtdIns may then be phosphorylated stepwise to PtdInsP and $PtdInsP_2$. $InsP_3$, derived by the hydrolysis of $PtdInsP_2$, is eventually dephosphorylated to yield free *myo*-inositol (Majerus, 1992). Two mechanisms maintain cellular *myo*-inositol levels: (1) de novo synthesis from glucose 6-phosphate catalyzed by *myo*-inositol 1-phosphate synthase; and (2) uptake of circulating *myo*-inositol via a non-saturable, energy-independent process, or a saturable, Na^+-dependent process (Sherman, 1989, for review). The relative contributions of these pathways in the inner ear are not known.

The significant decrease in the *myo*-inositol concentration suggests an age-dependent inability to maintain the endogenous *myo*-inositol pool. Although the mechanisms underlying this decrease remain to be established, the fact that *myo*-inositol content in aged human brain is half the level in young brain (Stokes et al., 1983) corresponds with our observations. Furthermore, tissue levels of the added *myo*-[^{3}H]inositol were higher in aged CSE and VSE. While this may suggest changes in an active transport mechanism, it may also reflect an altered equilibrium due to lower endogenous *myo*-inositol. In any case, a decreased *myo*-inositol content coupled with increased uptake of *myo*-[^{3}H]inositol would result in a precursor pool of higher specific radioactivity and consequently, in higher radioactivity in lipids and InsPs.

Depletion of cellular *myo*-inositol may in itself induce pathological changes. The participation of *myo*-inositol in osmoregulation and volume control is particularly interesting in the context of inner ear dysfunction. The role of outer hair cells as modulators of the transduction process may be intimately linked to proper maintenance of their intracellular pressure. The cytoplasm of outer hair cells is hyperosmotic but the responsible osmotic agent is unknown (Brownell, 1990). Turgor in these cells in part sustains their shape and their coupling to micromechanical events on the basilar membrane. Loss in turgor is associated with diminished electromotility (Brownell and Shehata, 1990) which in

turn may compromise tuning and sensitivity in the auditory periphery. Slow shape changes in response to elevation of intracellular calcium may also depend on intracellular pressure (Dulon et al., 1990). The role of turgor in cells of the VSE is not known but we can speculate that proper function of the vestibular apparatus also requires intact mechanisms of pressure homeostasis. If *myo*-inositol participates as an organic osmolyte in the maintenance of hair cell turgor, then its depletion should result in the subtle pathophysiological changes that are observed in aging animals.

In summary, *myo*-inositol metabolism is crucial for cell physiology as a constituent of volume-regulating and second messenger systems. The decreased *myo*-inositol content in aged rats suggests impaired *myo*-inositol synthesis or uptake, possibly compromising osmoregulation in the sensory epithelia. It remains open whether the limited availability of *myo*-inositol additionally leads to compensatory mechanisms of lipid metabolism and transmembrane signaling. Such changes would presently be masked by the differences in the *myo*-inositol precursor pool. Further study will be needed to establish the effects of aging on the molecular mechanisms regulating hair cell turgor, calcium homeostasis and motility in CSE and VSE.

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