Putative neurotransmitters in the rat cochlea at several ages

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We have performed a longitudinal study of the content of the putative neurotransmitter substances, enkephalin, dynorphin, and acetylcholine (ACh), in the cochlea of the Fischer 344 rat. It is the first study of transmitters in the rat cochlea over an extended time span. This study also provides biochemical verification of the presence of ACh in cochlear tissues. No change was seen in the cochlear content of these transmitter candidates up to 24 months of age.

In the past several years it has become clear that there are anatomically and chemically distinct efferent systems innervating the cochlea; the lateral system, which terminates on eight nerve dendrites under inner hair cells, and the medial, which directly innervates outer hair cells¹⁴,³⁰,³¹. The lateral efferents of species studied to date are known to contain enkephalins, dynorphins, calcitonin gene-related peptide and acetylcholine (ACh), while in the medial system only ACh has so far been identified as a putative transmitter substance¹-¹²,¹⁶,¹⁸-²⁰,²³,²⁴.

A considerable body of evidence has been developed supporting a role for acetylcholine as the transmitter of the efferent fibers arising in the superior olivary region which innervate the cochlea. Acetylcholine has been identified by bioassay in one mammalian cochlea²⁸, and enzymes related to the synthesis and degradation of ACh have been similarly localized by histochemical and biochemical means¹,³,⁵,¹³,²¹. Acetylcholine has also been shown to mimic in certain respects electrical stimulation of the cochlear efferents⁸.

Both proenkephalin- and prodynorphin-derived peptides have been identified in cochlear tissues and fluids, and have been localized by immunocytochemical, histochemical and biochemical techniques to the olivocochlear efferent fibers innervating the cochlea¹-⁷,⁹,¹²,¹⁶,¹⁸-²⁰,²³. These include enkephalins, dynorphins and neo-endorphins. Opioid receptors as well have been reported in the cochlea¹⁰,²⁰. Enkephalin- and dynorphin-like immunoreactivities have also been seen in the same cells in the superior olivary region¹,⁷, as have opioid peptide-like and choline acetyltransferase-like immunoreactivity¹,³, and enkephalin-like immunoreactivity and acetylcholinesterase activity⁵.

Acetylcholine and opioid peptides appear to serve as neurotransmitters or neuromodulators of the superior olivary fibers in the cochlea. The manner in which they may interact with each other, and the reasons for their co-localization at these synapses, have not yet been elucidated. In the course of studying these neuroactive substances we have determined their levels in the rat cochlea at different stages in the life span. Fischer 344 rats at 3, 12 and 24 months of age were used, representing the mature adult, old adult, and the extreme of the normal life span, respectively.

The combined high-performance liquid chromatography–radioimmunoassay (HPLC–RIA) for enkephalins has been described in earlier publications¹⁷,¹⁸. Whole cochleas were sonicated in 7.5 mM

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trifluoroacetic acid in 25% acetonitrile and centrifuged. Supernatants were either chromatographed for enkephalins and dynorphin, or lyophilized directly in a vacuum centrifuge. Dynorphin B chromatographed as a single sharp peak using the same HPLC method as used for enkephalins. Dynorphin was assayed as dynorphin B (rimorphin) using a commercially available kit (Peninsula Labs). This antiserum does not cross-react with other dynorphins, endorphins or dynorphins, and so results were the same whether or not samples were chromatographed prior to assay. Acetylcholine was assayed using the radiochemical method of McCaman and Stetzler as modified by Marchi et al.

The results are summarized in Table I. These data demonstrate the presence of Met-enkephalin, dynorphin B and ACh in the rat cochlea. No significant changes in levels of these putative transmitter substances are seen at the different ages studied.

Enkephalin-like, dynorphin-like and choline acetyltransferase-like immunoreactivities have previously been reported to be co-localized in lateral olivocochlear cell bodies in the rat. Although 3 classes of putative neurotransmitters are identified in the rat cochlea in this study, no change in their levels with age was seen. As auditory testing was not performed on these animals, it is possible that a deficit in auditory perception and cochlear transmitters may occur together at ages later than those studied. Hair cell losses have been reported to increase in the rat cochlea at even later ages. However, aging changes in auditory perception have been reported in Fischer 344 rats with an average age of 25 months, which is the normal lifespan of these animals. Also, some pronounced differences in the aging of the rat and human cochleas have been noted, which may make the rat a less desirable model for studying neural mechanisms of auditory aging.

The functions of these olivocochlear fiber systems are not clear at this time, in spite of much research, but are known to involve some inhibitory activity (see review by Wiederhold). Nieder and Nieder reported that efferent stimulation increases the ability to detect sound stimuli in a noisy background, by increasing the signal:noise ratio. This discriminative ability rapidly declines with age, and is greatly impaired in neural presbycusis. It is well known that hair cells in many species decline in number or demonstrate damage with increasing age. However, nothing is known to date about anatomical changes in the olivocochlear efferent neurons in aged animals of any species, although such information would be of great value in understanding the expression in the auditory system of the aging process.

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### Table I

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<thead>
<tr>
<th></th>
<th>3 Months</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
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<tbody>
<tr>
<td>Enkephalin</td>
<td>5</td>
<td>675 ± 95</td>
<td>733 ± 129</td>
</tr>
<tr>
<td>Dynorphin</td>
<td>5</td>
<td>54 ± 6</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>5</td>
<td>1340 ± 231</td>
<td>1290 ± 398</td>
</tr>
</tbody>
</table>

ase-positive cells in the guinea pig lateral superior olivary complex that project to the cochlea, *Neuroscience*, 9 (1983) 621–630.


