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Origin and immunolesioning of cholinergic basal forebrain innervation of cat primary auditory cortex

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Abstract

Numerous studies have implicated the cholinergic basal forebrain (cBF) in the modulation of auditory cortical responses. This study aimed to accurately define the sources of cBF input to primary auditory cortex (AI) and to assess the efficacy of a cholinergic immunotoxin in cat. Three anaesthetized cats received multiple injections of horseradish-peroxidase conjugated wheatgerm-agglutin into physiologically identified AI. Following one to two days survival, tetramethylbenzidine histochemistry revealed the greatest number of retrogradely labeled cells in ipsilateral putamen, globus pallidus and internal capsule, and smaller numbers in more medial nuclei of the basal forebrain (BF). Concurrent choline acetyltransferase immunohistochemistry showed that almost 80% of the retrogradely labeled cells in BF were cholinergic, with the vast majority of these cells arising from the more lateral BF nuclei identified above. In the second part of the study, unilateral intraparenchymal injections of the cholinergic immunotoxin ME20.4-SAP were made into the putamen/globus pallidus nuclei of six cats. Immuno- and histochemistry revealed a massive reduction in the number of cholinergic cells in and around the targeted area, and a corresponding reduction in the density of cholinergic fibers in auditory cortex. These results are discussed in terms of their implications for investigations of the role of the cBF in cortical plasticity.

Keywords: AI; Acetylcholine; Immunohistochemistry; Immunotoxin; Retrograde

1. Introduction

Numerous studies have implicated acetylcholine (ACh) in the modulation of sensory and motor cortical responses and functional organization. In the auditory system, application of ACh or ACh agonists to auditory cortex of cats has been shown to alter spontaneous and/or tone-evoked responses of individual cortical cells (e.g., McKenna et al., 1988). When paired with tonal stimulation, application of cholinergic agents (McKenna et al., 1989; Metherate and Weinberger, 1989, 1990) or of acetylcholinesterase (AChE) inhibitors (Ashe et al., 1989)

Abbreviations: ACh, Acetylcholine; AChE, acetylcholinesterase; AES, anterior ectosylvian sulcus; AI, primary auditory cortex; BF, basal forebrain; Ca, caudate nucleus; CAP, compound action potential; cBF, cholinergic basal forebrain; CF, characteristic frequency; ChAT, choline acetyltransferase; ChAT-ir, ChAT-like immunoreactivity; DAB, diaminobenzidine; DBh, horizontal division of the diagonal band of Broca; DBv, vertical division of the diagonal band of Broca; GAD, glutamic acid decarboxylase; GP, globus pallidus; HA, hypothalamic area; HRP, horseradish-peroxidase; HRP-WGA, horseradish-peroxidase conjugated wheatgerm-agglutin; IC, internal capsule; NAm, nuclei of the amygdala; PB, phosphate buffer; PBS, phosphate buffered saline; PBS+T, PBS containing Triton-X detergent; PES, posterior ectosylvian sulcus; Pu, putamen; $p75^{LNTR}$, p75 low-affinity neurotrophic receptor; p75-ir, $p75^{LNTR}$-like immunoreactivity; RF, receptive field; SI, substantia innominata; SMN, medial septal nucleus; SOJ, septo-olfactory junction; SSS, suprasylvian sulcus; TMB, tetramethylbenzidine

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alters the receptive-field (RF) properties of cortical cells in a frequency-specific manner, suggesting that endogenous ACh plays a modulatory role in reshaping the RFs of auditory cortical cells. Although it is now widely accepted that mammalian neocortex derives its primary cholinergic input from the basal forebrain (BF), the sources of cholinergic BF (cBF) projections to the primary auditory cortex (AI) have not been accurately characterized.

One of the first reports of striato-cortical projections in the cat was that of Jayaraman (1980), who made large injections of horseradish peroxidase (HRP) into anatomically defined AI and found retrogradely labeled cells in the putamen (Pu), globus pallidus (GP), internal capsule (IC) and ventrally in the caudate nucleus (Ca). These results were confirmed by observations of similar patterns of labeling following injection of fluorescent retrograde tracers into anatomically defined AI (Oleshko and Maisky, 1993). Other studies, however, have indicated that BF projections to the auditory cortex are not restricted to the striatum and paleostriatum, and Irle and Markowitsch (1984) reported that single injections of HRP into anatomically defined auditory cortex labeled cells in the vertical division of the diagonal band of Broca (DBv) and the substantia innominata (SI).

Given the fact that the location of AI relative to sulcal landmarks varies between animals (e.g., Reale and Imig, 1980), a limitation of these retrograde studies has been the failure to identify AI physiologically. In the only report to employ physiological definition of injection sites, single injections of HRP-conjugated wheatgerm-agglutin (HRP-WGA) into cat AI (12-kHz region in one cat, 25-kHz region in another) labeled large cells in the horizontal division of the diagonal band of Broca (DBh) and in SI (Rouiller et al., 1989); no cells were reported in Pu, GP or IC nuclei.

A further limitation in most of the connectivity studies is that cellular morphology and distribution have been the only criteria used to establish the cholinergic nature of retrogradely labeled cells. Such criteria are inadequate, as it has been found that cholinergic and GABAergic cells of BF share very similar characteristics and overlapping spatial distributions (Fisher et al., 1988). The distribution of putative cholinergic cells and fibers in BF and neocortex is typically described according to the localization of AChE (the ACh hydrolytic enzyme) or choline acetyltransferase (ChAT – the ACh synthesizing enzyme). ChAT immunohistochemistry provides the most sensitive and accurate tool presently available for the identification of cholinergic structures in various species (Oda, 1999), and the central cholinergic system of the cat has been extensively mapped using the ChAT immunohistochemical method (Kimura et al., 1981). Only one study has reported histochemically identifying the BF projections to cat auditory cortex. Parent et al. (1981) used the less convincing AChE marker of cholinergic activity, and reported that injections of tracer into anatomically defined AI retrogradely labeled AChE-positive cells in GP, Pu and SI. The first aim of the present research was therefore to accurately identify and characterize the cholinergic BF inputs to physiologically identified AI using retrograde tracing and immunohistochemical techniques. A preliminary report of this work has been presented in abstract form (Kamke et al., 2004a).

A second aim relates to investigations of the putative role of the BF cholinergic system in various forms of learning and plasticity. As well as employing ACh agonists and antagonists, the potential role of the cBF in cortical plasticity has been investigated in various paradigms employing electrical stimulation and/or excitotoxic lesions of BF. A major limitation with these techniques is that their effects are not restricted to cholinergic neurons. In rodents, it has been possible to selectively lesion cholinergic cells in BF using the immunotoxin 192IgG-SAP, which is a conjugation of an antibody raised against the rat p75 low-affinity neurotrophic receptor ($p75^{LNTR}$) and Saporin, a ribosome inactivating protein (for review, McGaughy et al., 2000). Although the 192IgG-SAP immunotoxin does not identify $p75^{LNTR}$ in non-rodent species, an analogous antibody to the human $p75^{LNTR}$ (clone ME20.4) has recently been found to identify the majority of ChAT-positive cells in fixed tissue of the cat (Tremere et al., 2000). This antibody has also been conjugated to Saporin, and the ME20.4-SAP immunotoxin has been used to selectively lesion cholinergic cells in BF of primates (e.g., Fine et al., 1997), sheep (Ferreira et al., 2001) and rabbit (Beach et al., 2000).

In the study of cholinergic function and dysfunction, it is apparent that immunotoxins provide one of the most discrete tools available for investigating cholinergic influences. The second aim of the present study was to investigate the effectiveness of the ME20.4-SAP immunotoxin in cat. To this end, the area identified in the retrograde study as providing the primary source of cholinergic input to AI was targeted with intraparenchymal injections of the immunotoxin. This paradigm was employed in favor of intraventricular infusions in order to not only provide evidence of the in vivo affinity of the toxin, but to also allow for a description of the discreteness of any lesion produced.

2. Materials and methods

All surgical and experimental procedures were performed in an electrically shielded, sound-attenuating room using procedures approved by the Monash University Department of Psychology Animal Ethics Committee. Three adult domestic cats with normal hearing were used in the retrograde tracing study, and immunotoxic