Evidence for direct cortical innervation of medial olivocochlear neurones in rats

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Received 19 June 1999; received in revised form 11 November 1999; accepted 2 February 2000

Abstract

We have investigated the morphological relationship between auditory cortex efferents and medial olivocochlear neurones. Using combined retrograde and anterograde tracing we describe close contacts between medial olivocochlear neurones and corticofugal terminals in the ventral nucleus of the trapezoid body. The data indicate a possible direct action of the auditory cortex on the activity of the medial olivocochlear neurones and thus possibly the sensitivity of the cochlea. © 2000 Elsevier Science B.V. All rights reserved.

Key words: Hearing; Auditory cortex; Immunocytochemistry; Anterograde labelling; Retrograde labelling; Olivocochlear efferent

1. Introduction

Not only can the auditory cortex be considered the final target of ascending auditory information, it is also the source of descending pathways. The two largest and most extensively described corticofugal projections terminate in the medial geniculate complex and the inferior colliculus (Beyerl, 1978; Coleman and Clerici, 1987; Winer and Larue, 1987; Games and Winer, 1988; Roger and Arnault, 1989; Arnault and Roger, 1990; Herbert et al., 1991; Saldanha et al., 1996). Recently, the use of an anterograde tracer has demonstrated smaller but direct projections from the auditory cortex to the auditory brainstem nuclei, i.e. the superior olivary complex and the cochlear nucleus (Feliciano et al., 1995). A subsequent retrograde tracing study has shown that the descending projection to the cochlear nucleus originates in layer V of the auditory cortex (Weedman and Ryugo, 1996).

The projection from the auditory cortex to the superior olivary complex appears to terminate predominately in the ventral nucleus of the trapezoid body (Feliciano et al., 1995). This subdivision of the superior olivary complex contains, amongst other neurones, the medial olivocochlear neurones, which innervate the outer hair cells in the organ of Corti (White and Warr, 1983; Vetter et al., 1991; Vetter and Mignaini, 1992). This auditory efferent pathway is thought to have an inhibitory effect on cochlear neural output (Warren and Liberman, 1989). In large part this appears to be the result of an action of the medial olivocochlear neurones on the outer hair cell ‘active process’ that determines cochlear micromechanical behaviour (Patuzzi and Robertson, 1988).

In the present study we have investigated whether the auditory cortex efferents directly innervate the medial olivocochlear neurones in the ventral nucleus of the trapezoid body. For this purpose we combined a retrograde tracer injection in the cochlea with an anterograde tracer injection in the auditory cortex, visualising the medial olivocochlear neurones and the terminal processes of auditory cortex efferents within the same sections. To determine more precisely which areas of the auditory cortex project to the superior olivary complex, and the ventral nucleus of the trapezoid body in particular, we also made a number of retrograde tracer injections aimed at the ventral nucleus of the trapezoid body and investigated the pattern of labelled neurones within the auditory cortex.

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2. Materials and methods

All experimental protocols conformed to the Code of Practice of the National Health and Medical Research Council of Australia and were approved by the Animal Experimentation Ethics Committee of the University of Western Australia. Under deep anaesthesia with sodium pentobarbital (6 mg per 100 g body weight, intraperitoneally) 11 male Wistar rats (190–400 g) received an injection of fluorogold (FG) in the right cochlea, immediately followed by an injection of biotinylated dextran amine (BDA) in the left auditory cortex, both during one experimental procedure. Using the same anaesthesia regime, seven other male Wistar rats (190–230 g) received an iontophoretic injection of FG in the superior olivary complex.

For intracochlear injection, glass pipettes were filled with 1% FG (Fluorochrome) in 0.9% saline and connected to a Hamilton syringe. The cochlea was exposed and two small holes were drilled, one in the apex and one in the first turn of the scala tympani, after which approximately 3 µl FG was slowly injected in the first turn hole. The holes were sealed with small pieces of gelfilm to prevent post-operative leakage and the wound was sutured.

For retrograde tracing with FG from the superior olivary complex we prepared glass pipettes with a tip diameter of 119–220 µm. After injection, the pipette was left in place for 5 min and then removed in small steps to minimise leakage of BDA along the pipette track. The wound was then sutured and rats received post-operative buprenorphine (0.003 mg/100 g body weight) as analgesic. Survival times varied from 5 to 7 days.

For retrograde tracing with FG from the superior olivary complex we prepared glass pipettes with a tip diameter of 30–60 µm, which were filled with 1% FG (Fluorochrome) in 0.1 M acetic acid buffer. After anaesthesia rats were placed in a stereotaxic apparatus and iontophoretic injections of FG (5 µA, 5 s on/off, 7 min) were made. The co-ordinates of the superior olivary complex were determined using the atlas of Paxinos and Watson (1982). The pipette was left in place for 5 min following injection and then removed in small steps to reduce leakage along the pipette track. Survival time of these rats was 3 days.

After the appropriate survival times, rats were again deeply anaesthetised with pentobarbital and transcranially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After perfusion the brains were removed, postfixed overnight and saturated in 30% sucrose in PB at 4°C. Transverse 40 µm sections were cut on a freezing microtome (Kryomat 1703, Leitz) and collected in PBS.

For the double labelling experiments (BDA and FG) several series were cut. One series of each experiment was mounted on gelatine-coated slides for counterstaining with toluidine blue. The remaining series were stained free-floating for BDA and FG. Before staining sections were rinsed 10 min in 3% H2O2 in methanol to eliminate endogenous peroxidase. In between incubations, sections were rinsed three times with PBS for 20 min each. For visualisation of BDA sections were preincubated for 1 h in PBS containing 0.1% BSA and 0.3% Triton X-100 and then incubated for 2 h in ABC (Vector ABC kit 1:800 in PBS). Subsequently, sections were developed with 0.02% 3,3’-diaminobenzidine tetrahydrochloride (DAB) with 0.2% ammonium nickel sulphate (Ni) and hydrogen peroxide. This resulted in blue-black BDA fibres. For visualisation of FG the same sections were then pre-incubated for 1 h in PBS with 0.1% BSA, 0.2% Triton X-100 and 5% normal goat serum (incubation solution), followed by 3 days incubation at 4°C with a rabbit FG antibody (Chemicon) diluted 1:18000 in the incubation solution. Sections were then incubated for 90 min in biotinylated goat anti-rabbit (Sigma, 1:1000 in incubation solution) and 2 h in ABC. Finally, sections were treated with DAB as described above but without the addition of Ni. This procedure resulted in brown FG labelling. Sections were mounted on gelatine-coated slides, dried overnight, dehydrated and coverslipped with DePeX.

For the FG tracing experiments from the superior olivary complex 40 µm sections were cut into two series and every fifth section per series was collected in PB. One series of each experiment was mounted immediately on gelatine-coated slides and dried overnight at
room temperature for counterstaining with toluidine blue followed by dehydration in a graded series of ethanol and coverslipping via xylene in Depex. The other series was also mounted immediately on gelatinised slides, then dehydrated and coverslipped without counterstain.

Double labelled sections were examined with an Olympus Vanox-2 microscope and a 100× oil immersion objective. Photographs were made with an Olympus DP10 Microscope Digital Camera System. The only adjustments made to the digital images were changes in brightness and contrast.

The FG-only labelled sections were analysed using an Olympus Vanox-T AH-2 fluorescence microscope. The location of the auditory cortical areas was confirmed by comparison with the adjacent toluidine blue-stained sections.

3. Results

3.1. Double labelling experiments

Fig. 1A shows schematically the experimental arrangement of the double labelling experiments and Fig. 1B–D illustrates the typical distribution and appearance of neurones in the superior olivary complex after intracochlear injection. Intracochlear FG injection resulted in labelling of the lateral and medial olivocochlear neurones within the ipsilateral lateral superior olive (LSO) and ipsi- as well as contralateral ventral nucleus of the trapezoid body (VNTB). The iontophoretic injections with the anterograde tracer BDA, aimed at the auditory cortex, varied from 0.5 to 1.7 mm in their medio-lateral diameter, from 1 to 3.4 mm in dorso-ventral direction and from 0.6 to 2 mm in rostro-caudal direction. All injections included the deep layers of the cortex. The precise location of injections was determined with the help of the atlas of Swanson (1992). Light microscopical analysis showed that six injections were mainly located in the primary auditory cortex, two injections were half in the primary auditory cortex and half in the ventral temporal association areas and one injection (H88) predominantly in the ventral temporal association areas. Two injections were localised outside these cortical areas, one injection rostral to the primary auditory cortex. These latter two injections did not result in anterogradely labelled fibres within any of the auditory brain structures investigated. The location of the nine injections within the auditory areas, i.e. the primary auditory cortex and/or the ventral temporal association areas, is illustrated in Fig. 2A. The following description is restricted to the results of these nine injections.

All injections resulted in labelled fibres in the medial geniculate complex and inferior colliculus. These projections have been described in detail by others (Beyerl, 1978; Coleman and Clerici, 1987; Roger and Arnault, 1989; Arnault and Roger, 1990; Herbert et al., 1991; Saldaña et al., 1996) and will therefore not be discussed further. All injections, including H88, which was predominantly localised in the ventral temporal association areas (Fig. 2A), resulted in labelled fibres in and around the superior olivary complex. The majority of labelled BDA fibres within the superior olivary complex was found ipsilateral to the cortical injection site with only few fibres located contralaterally. Many BDA fibres were observed coursing in the trapezoid body, ventral and medial to the superior olivary complex, as well as in the pyramidal tract. These fibres showed very few varicosities. Close examination also revealed BDA-labelled processes within the VNTB, especially the ventral part of this nucleus. Whether these fibres were collaterals from the fibres coursing through the trapezoid body or were separate projections could not be established with certainty. The number of labelled fibres in the VNTB was low and not many varicosities were observed. Many fibres seemed to be passing through the VNTB without contacting any neurones or dendrites in this subdivision of the superior olivary complex. Because of the limited number of fibres observed we were unable to confirm any topographical distribution of the cortical innervation, as described by Feliciano et al. (1995).

Despite the small number of fibres present in the VNTB, very close proximity of BDA-labelled cortical fibres with FG-labelled medial olivocochlear neurones was regularly observed (Fig. 2B–D). Such close proximity was regarded as a putative contact when it involved a close apposition of a BDA-labelled fibre with varicosities and a FG-labelled structure within the same focal plane. Contacts were limited to the olivocochlear dendrites only and were never observed on the somata.
of the medial olivocochlear neurones. We also observed BDA-labelled fibres with varicosities that were intermingled with the dendrites of medial olivocochlear neurones in the VNTB but not in close apposition to these dendrites.

3.2. Retrograde labelling of cortico-olivary efferents

Iontophoretic injections with FG resulted in small to large injection sites, spreading over 400–1000 μm in rostro-caudal direction. The injection sites typically showed a small necrotic core, surrounded by an area of heavily labelled cell bodies and a peripheral halo of lightly labelled neuropil. In three animals the core of the injection was restricted to the VNTB itself. In only one of these, an animal with an injection located in the centre of the rostro-caudal extent of the VNTB, were small numbers of FG-labelled cells observed in layer V of the ipsilateral primary auditory cortex. In the remaining four animals, larger injections were made which encompassed most of the VNTB and also spread to other areas including the medial nucleus of the trapezoid body, superior periolivary nucleus, medial and lateral superior olive. In these latter animals more numerous labelled neurones were seen in primary auditory cortical areas compared to those animals in which injections were limited to only part of the VNTB. Two of these animals with larger injections also exhibited labelled cells in the contralateral auditory cortex, and one showed labelled cells in the ventral temporal association areas as well as in the primary auditory cortex. The number and distribution of labelled cells in the auditory cortex and the ventral temporal association areas of this latter animal is illustrated in Fig. 3.

4. Discussion

This paper confirms the existence of a direct bilateral projection to the VNTB as described by Feliciano et al. (1995), although we were unable to confirm any topographical distribution of labelled fibres and terminals. Compared to the earlier study, the amount of labelled fibres we observed in the VNTB was small. However, as can be seen in the reconstructed lateral surface view of the present injections their size and spread within the cortex was small, which might explain the limited number of fibres observed. The small injections are most likely due to the fact that we used a lower concentration of biotinylated dextran and a shorter iontophoresis procedure than Feliciano and coworkers. The limited number of labelled fibres within the VNTB is also not unexpected in view of the results of retrograde tracing with FG from the superior olivary complex. In the seven brains we investigated, we found only very small numbers of labelled neurones in the auditory cortex,
even after large FG injections in the superior olivary complex. This indicates only a very small and diffuse input from the auditory cortex to the superior olivary complex. Interestingly, in one brain, with a large FG injection in the superior olivary complex spreading over several subdivisions including the VNTB, we also observed labelled neurones in the ventral temporal association areas. These data suggest some input from these areas. This is in agreement with the present results of anterograde labelling, since the small injection (H88) that was predominantly located in the ventral temporal association areas also resulted in labelled fibres in the VNTB.

Despite limited numbers of corticofugal fibres in the VNTB we did observe very close proximity of fibres and varicosities with the medial olivocochlear neurones, which is suggestive of synaptic contacts (Wouterlood and Groenewegen, 1985), although electron microscopy would be necessary to prove the existence of such synapses. Our observations indicate that there may be a direct cortical innervation of the medial olivocochlear system and it is possible that a direct influence of auditory cortical areas on cochlear functioning exists. The cortical input to the VNTB and its olivocochlear neurones seems very limited when compared to the input arising from the inferior colliculus (Caicedo and Herbert, 1993; Thompson and Thompson, 1993; Vetter et al., 1993). However, we cannot be certain that we labelled all olivocochlear neurones nor that we labelled the whole cortical projection in each animal, and hence we cannot provide any quantification of our results.

Although this study shows there is a direct cortical input to the medial olivocochlear system, other immunohistochemical and physiological studies are necessary to establish the function of such innervation, by determining the neurotransmitters involved and the possible effects on the outer hair cells and the sensitivity of the cochlea. Auditory corticofugal fibres with varicosities have also been reported in the small cell cap of the cochlear nucleus (Feliciano et al., 1995). We were able to confirm this in the present study (data not shown). This subdivision of the cochlear nucleus is a preferential target of the type I low spontaneous rate primary afferents (Lieberman, 1993; Tsuji and Liberman, 1997) and has been hypothesised to play a role in encoding stimulus intensity (Winter et al., 1990; Ghoshal and Kim, 1996). This region is also thought to be involved in feedback to the medial olivocochlear system (Kim et al., 1995) via input from type II primary afferents from the outer hair cells, as well as itself receiving input from medial olivocochlear neurone collaterals (Ryan et al., 1990; Benson et al., 1996). It is thus possible that the medial olivocochlear neurones in the VNTB and the targets of their collateral projections in the cochlear nucleus receive similar and parallel inputs from the auditory cortex.

Acknowledgements

Supported by grants from the National Health and Medical Research Council, the Medical Research Infrastructure Fund and The University of Western Australia.

References


Saldana, E., Feliciano, M., Mugnaini, E., 1996. Distribution of descending projections from primary auditory neocortex to inferior