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An investigation of dual innervation of the temporomandibular joint and external ear canal of the rat

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SUMMARY

Patients suffering from temporomandibular joint disease may experience referred otalgia, or pain localised to the ear. The aim of this study was to determine if afferent fibers from the temporomandibular joint and the external acoustic meatus of the rat converge to a single afferent perikaryon in the semilunar ganglion of the trigeminal nerve. In anaesthesised animals Fluorogold was injected into either the temporomandibular joint cavity or the external ear canal and True Blue was administered to the other location in Sprague Dawley rats. Ten to fourteen days later the animals were anaesthesised and the trigeminal ganglia removed for cryostat sectioning and fluorescence microscopy. A small proportion of nerve fibers supplying the temporomandibular joint and the external ear canal converge to single perikarya within the semilunar ganglion of the trigeminal nerve of the rat. This does not occur in a sufficiently high degree to account for the phenonomen of referred otalgia caused by a primary pathology in the temporomandibular joint.

Key Words: External ear canal - temporomandibular joint - trigeminal glanglion - Fluorogold - True Blue.

INTRODUCTION

Referred pain is a phenomenon in which primary pathology arising in visceral or deep somatic structures results in pain felt mainly or exclusively in surface regions of the body (Brodal, 1981). Thus, patients suffering from temporomandibular joint disease may experience referred otalgia, or pain localised to the ear (Blake et al., 1982; Luz et al., 1997).

The temporomandibular joint is innervated by somatic sensory fibres in the auriculotemporal, masseteric and posterior deep temporal nerves. The cell bodies of these fibres are located within the trigeminal ganglion (Romfh et al., 1979). The external ear canal has a complex somatic sensory innervation, receiving a supply from the auriculotemporal and greater auricular nerves, and from the auricular branch of the vagus, also known as Arnold's nerve (Grey, 1995). The neuronal cell bodies are within the trigeminal, geniculate, glossopharyngeal and jugular vagal ganglia (Folan-Curran et al., 1994). It was noted that nerves supplying both the temporomandibular joint and the external ear canal had cell bodies within the trigeminal ganglion. It was proposed that this was a locus of origin for referred pain in keeping with Sinclair et al. (1947), who suggested that the peripheral branch of a spinal dorsal root or a cranial ganglion cell could dichotomise, giving branches to two non-continuous receptive fields.

The objective of this study was to determine whether there is dichotomisation of nerve fibers to the external acoustic meatus and the temporomandibular joint from single neurons in the trigeminal ganglion. The method of study involved the application of one fluorescent retrogade tra-

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cer dye, such as Fluorogold to the external ear canal, while another dye, True Blue, would be injected into the temporomandibular joint cavity. Colocalisation of both tracers within a single ganglionic cell would indicate dichotomisation. This method has previously been used by Mc Neill and Burden (1986) to demonstrate convergence of sensory processes from the heart and left ulnar nerve onto a single afferent perikaryon.

Materials and Methods

Young adult Sprague-Dawley rats (N=13) 150-450g free of otitis externa were used. All procedres were carried out in accordance with the Cruelty to Animals Act, 1876. The left external ear canal was examined with a Carl-Zeiss operating microscope (x16). The epithelium of the inner bony portion was then abraded with silver nitrate to facilitate dye uptake (Tierney et al., 1993). Fluorogold or True Blue was applied to the external ear canal and the other dye was injected into the temporomandibular joint in nine experimental animals. An aliquot of Fluorogold (Fluorochrome Inc., Colorado) 3% w/v in water and/or True Blue (Sigma) 1% w/v in glacial acetic acid was applied to the prepared surface with a Hamilton syringe; the amount used was either 2 or 4 µl, to observe the effect of varying the dye quantity. The animals were then deeply anaesthetised with 2.5% 2,2,2-tribromoethanol (1 ml/100g body weight ip). The left temporomandibular joint was surgically exposed; Fluorogold and/or True Blue was injected into the upper compartment of the joint cavity. The joint capsule was sealed with superglue to minimise dye leakage and the wound was sutured. Both dyes were administered simultaneously to either the temporomandibular joint or the external ear canal in four other animals to obtain positive controls for double staining. The animals were allowed to survive for 7-10 days to allow dye transport to the neuronal cell bodies. The animals were then reanaesthetised with ether and a lethal dose (2.5 ml/100g body weight ip) of 7.5% w/v chloral hydrate. They were perfused with 180ml of 0.1M phosphate buffer (pH 7.4) containing 10% formalin v/v and 18% sucrose w/v through the left ventricle. The trigeminal ganglia from both sides were dissected out from the cranial cavity (those on the left were experimental; those on the right served as controls for systemic spread of dye). The brainstem was also removed to allow examination of the motor nucleus of the trigeminal nerve, which would control for spread of dye from the temporomandibular jont cavity to the musculature surrounding the joint. Specimens were stored overnight in fixative at 4°C. 20 µm sections were cut with a cryostat, thaw-mounted onto glass slides and cover-slipped. Initially, every fifth section was examined; subsequently, serial sections were viewed with a Leitz fluorescence microscope D filter system (excitation wavelength 355-425 nm; suppression filter 460 nm).

Results

Following the application of different tracer dyes to the temporomandibular joint and the external ear canal, numerous singly stained cells were observed within the trigeminal ganglion, clustered laterocaudally around the origin of the mandibular division. These cells were readily distinguishable from background autofluorescence. Fluorogold appears as brightly fluorescent yellow cytoplasmic granules (Fig. 1). True Blue forms a uniform brilliant blue (Fig. 2). In some of the animals, spread of the tracer dyes had occurred, as evidenced by staining within the motor nucleus of the trigeminal nerve in the brainstem (Fig. 2). The dye present was either that applied to the external acoustic meatus or that injected into the temporomandibular joint. Dual staining of neurons in the trigeminal ganglia taken from these animals was not considered signficant. In 3 of 4 animals without such leakage of dye, 2-3 dual stained cells, which were characterised by a lavender cytoplasm with gold vesicles, were observed in the trigeminal ganglion (Fig. 3). This constituted a very small proportion of the total number of stained cells. Of those control animals where both dyes had been applied to a single locus (either the external ear canal or the temporomandibular joint) numerous dual stained cells were observed. The appearance of dual stained cells was more distinctive in animals who had received 2 µl aliquots of dye, being lavender in tint. Where 4 µl quantities of dye were used, the colour of dual stained cells was nearly white, and harder to distinguish from neurons stained with Fluorogold alone.

Discussion

In this study, cells stained after dye injection into the temporomandibular joint and application of dye to the ear were intermingled in the laterocaudal portion of the ipsilateral trigeminal ganglion, around the origin of the mandibular nerve. This is in keeping with a report from Allen (1924) who identified two separate and distinct portions in the ganglion semilunare of the cat (the ophthalmic-maxillary and mandibular areas) and found that cells from the inferior alveolar and lingual nerves of the mandibular division are dis-

persed throughout the caudal division. Likewise, Marfurt (1981) demonstrated that cells of the mandibular division of the trigeminal nerve cluster posterolaterally; there was no clear demarcation observed between cells of the inferior alveolar and mental nerves.

A primary problem with the use of retrograde tracer dyes is the spread of such dye away from its site of application into the adjacent tissues; this was anticipated to occur from the temporomandibular joint and was controlled for by examining the motor nucleus of the trigeminal in the brainstem. Spread from the external ear canal was considered less likely, but was also shown to occur. Application of silver nitrate to the skin of the external ear canal abrades the epithelium but may, if it touches the tympanic membrane, increase the permeability of the tympanic membrane to such dyes (Tierney et al., 1993); thus, dye could have spread into the middle ear cavity and been taken up by trigeminal neurons innervating the tensor tympani muscle.

The exact appearance of neurons dual stained with True Blue and Fluorogold was previously unclear. Tierney et al. (1993) described that True Blue stained the cytoplasm uniformly blue, with separate Fluorogold vesicles easily discernable within it, while Schmued and Fallon (1986) stated that double stained neurons were pale lavendar or white. This study found that the appearance of dual stained neurons varied with the quantity of the dye used, being more definitive with small volumes of Fluorogold. The diagnostic feature of dual staining was a lavendar tint in the cytoplasm.

This study showed that some neurons were double labelled with True Blue and Fluorogold after application of one dye to the external ear canal and injection of the other into the temporomandibular joint cavity, demonstrating that convergence of primary afferent fibres from these sources to single sensory neurons does exist. However, the level observed was very low, suggesting little support for the dichotomisation theory of Sinclair et al. (1947). It has not been ascertained that all sensory fibres innervating these areas have been stained; indeed, for practical reasons, dye was injected only into the upper compartment of the temporomandibular joint cavity, while Kido et al. (1991) showed that there are also nerve fibres innervating the lower compartment. Therefore, a higher level of axonal convergence than has here been shown cannot be outruled.

Similar studies have been performed on other areas where referred pain is known to occur, both within and outside the trigeminal nerve system. A double labelling study between the supraorbital nerve and the perivascular plexuses of the anterior and middle meningeal

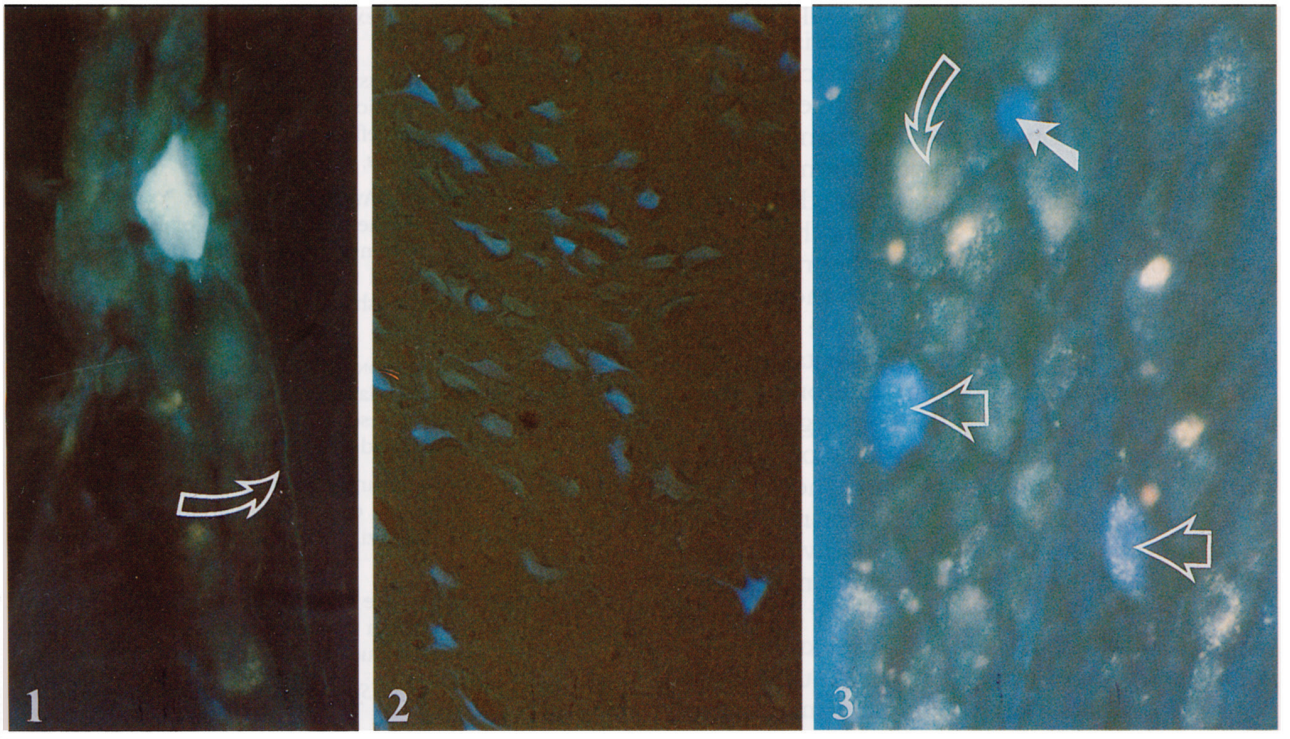

Fig. 1.– A unipolar sensory neuron and axon (curved arrow) in the trigeminal ganglion after Fluorogold application to the ipsilateral external ear canal. x 625.

Fig. 2.– Multipolar neurons of the trigeminal motor nucleus in the pons after leakage of True Blue from the ipsilateral temporomandibular joint. x 250.

Fig. 3.– Dual stained neurons (straight open arrows) in the left trigeminal ganglion after Fluorogold injection into the left temporomandibular joint and True Blue injection into the left external ear canal. Note adjacent neurons stained singly with Fluorogold (curved open arrow and True Blue (closed arrow). x 625.

arteries by Borges and Moskowitz (1983) resulted in virtually all of the labelled neurons containing one tracer alone. A similar study on convergence in the dorsal root ganglia between sensory fibres from the diaphragm and shoulder skin showed dual labelled cells representing 1% of the total number of stained cells (Laurberg and Sorenson, 1985). Thus, the general trend, from this study and the others described, implies that dichotomising peripheral fibres as proposed by Sinclair et al. (1947) exist in the rat, but they are a rare phenomenon; a finding that has also been borne out by physiological studies (Devor et al., 1984).

There has as yet been little research into the specific area of convergence between impulses from the temporomandibular joint and the external ear to second order sensory neurons in the trigeminal system in the brainstem. The central processes of all sensory fibres from both the temporomandibular joint and the ear terminate within the brainstem in the sensory nuclei of the trigeminal nerve (Bossy, 1968). Evidence from studies on afferent fibres from other deep somatic and visceral sites to neurons in the brainstem and the dorsal horn of the spinal cord show that convergence is a prominent phenomenon. A review of studies on visceral pain by Ness and Gebhart (1990) showed that more than 95% of neurons responsive to visceral stimuli in the spinal cords of a variety of species also received a cutaneous input. Sessle et al. (1986), demonstrated substantial convergence of cutaneous, tooth pulp, visceral, neck and muscle afferents onto nociceptive and non-nociceptive neurons in the trigeminal subnucleus caudalis; ascending projection of these neurons to the thalamus was also demonstrated, providing evidence supporting the convergence-projection (Ruch, 1946) theory of referred pain. It has also been shown that stimulation of craniofacial muscle afferents induces prolonged facilitatory effects in trigeminal nociceptive brain-stem neurons (Hu et al., 1992), suggesting that the convergence-facilitation model (MacKenzie, 1909) of referred pain may also be relevant. Thus, there is evidence supporting all of the mechanisms of referred pain outlined above. While individual ganglionic cells in the trigeminal ganglion may give peripheral processes which supply both the temporomandibular joint and the external ear, contributing to referred pain, this is unlikely to be the sole mechanism. Complete mapping of the sensory pathway as far as the cerebral cortex, with documentation of all sites of possible convergence of a signal from its locus of origin to the site at which it is consciously perceived is necessary.

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