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BRAIN RESEARCH

Research Report

Innervation of ventricular and periventricular brain compartments

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ABSTRACT

Synaptic transmission is divided into two broad categories on the basis of the distance over which neurotransmitters travel. Wiring transmission is the release of transmitter into synaptic clefts in close apposition to receptors. Volume transmission is the release of transmitters or modulators over varying distances before interacting with receptors. One case of volume transmission over potentially long distances involves release into cerebrospinal fluid (CSF). The CSF contains neuroactive substances that affect brain function and range in size from small molecule transmitters to peptides and large proteins. CSF-contacting neurons are a well-known and universal feature of non-mammalian vertebrates, but only supra- and subependymal serotonergic plexuses are a commonly studied feature in mammals. The origin of most other neuroactive substances in CSF is unknown. In order to determine which brain regions communicate with CSF, we describe the distribution of retrograde neuronal labeling in the rat brain following ventricular injection of Cholera toxin, ß subunit (CTß), a tracer frequently used in brain circuit analysis. Within 15 to 30 min following intraventricular injection, there is only diffuse, non-specific staining adjacent to the ventricular surface. Within 2 to 10 days, however, there is extensive labeling of neuronal perikarya in specific nuclear groups in the telencephalon, thalamus, hypothalamus and brainstem, many at a considerable distance from the ventricles. These observations support the view that ventricular CSF is a significant channel for volume transmission and identifies those brain regions most likely to be involved in this process.

1. Introduction

Neurons of the brain generally communicate with one another through the release of transmitters and other neuroactive substances at axon terminals to affect the function of various postsynaptic elements. For example, the predominant excitatory and inhibitory transmitters, glutamate and GABA, are released into short synaptic clefts, 12–20 nm in height (Savtchenko and Rusakov, 2007) and interact with nearby postsynaptic receptors (Bito, 2010; Lopez-Munoz and Alamo, 2009). Many other neuroactive substances are also released from axon varicosities but exert their effects at a distance through volume transmission (Agnati et al., 1986, 2010; Descarries and Mechawar, 2000; Fuxe et al., 2010; Okubo and Iino, 2011b). Volume transmission may take

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place over considerable distances, including transport via CSF, through extracellular spaces, and along fiber bundles (Bjelke et al., 1995; Fuxe et al., 2010). It is well known that normal production and flow of CSF are critical for optimal brain function (Johanson et al., 2011). Early work on CSF led to the conclusions that it is formed as a secretion by the choroid plexus in the lateral, 3rd and 4th ventricles and that it functions primarily in mechanical stabilization of the brain as well as nutrient and waste flow (Cushing, 1914; Oreskovic and Klarica, 2010). This view has been significantly revised as subsequent research conducted over many decades indicates that CSF is formed continuously by ongoing exchange with brain interstitial fluid as well as secretion from the choroid plexus and, after circulation through the ventricular compartments to subarachnoid space, is resorbed into cranial venous arachnoid villi and the olfactory lymphatic system (Pollay, 2010).

The CSF was not viewed as a conduit for neuronal communication until studies in the 1970s demonstrated both sub- and supraventricular plexuses of raphe serotonergic terminals (Aghajanian and Gallager, 1975; Chan-Palay, 1976; Lorez and Richards, 1975; Richards, 1977). The precise function of the serotonergic ependymal plexuses is unknown, but a recent report suggests that the subependymal plexus functions to modulate activity of the adult ventricular germinal epithelium (Jahanshahi et al., 2011). CSF-contacting neurons are more common in non-mammalian species (Vigh et al., 2004). On the other hand, mammalian ventricular CSF also contains a number of neuroactive substances, many of which have been shown to affect brain function, presumably by volume transmission (for reviews, see Johanson et al., 2008, 2011; Veening and Barendregt, 2010; Vigh et al., 2004). The source of these substances is presumed to be brain interstitial fluid but specific sources other than the serotonergic plexuses are not known. The present study is an examination of the disposition of the well-established tracer CTß (Ericson and Blomqvist, 1988; Trojanowski, 1983) following intraventricular infusion and therefore identifies specific areas of the brain that may provide some of these neuroactive substances in CSF.

2. Results

Six types of labeling are observed after intraventricular injections: 1) ependyma, including tanycytes; 2) perivascular spaces; 3) neuronal perikarya in nuclei with known supraependymal plexuses (dorsal raphe); 4) nuclei lying adjacent to the CSF compartment with axonal plexuses close to the ventricle (for example, the suprachiasmatic nucleus; Schwartz and Reppert, 1985); 5) neuron-containing circumventricular organs (subfornical organ); and 6) neuronal perikarya in nuclei not directly adjacent to the ependymal surface but with axon terminal fields within the range of diffusion of retrograde tracers and, hence, also a potential source of CSF neuroactive substances.

2.1. *Short-term versus long-term survival*

Bilateral labeling of the ependyma and associated cilia surrounding all ventricles is present within 15 min of injection (Fig. 1a). This labeling of the ventricular lining remains unchanged over the course of a week. Diffuse reaction product also extends into brain parenchyma around the ventricular system for a distance of up to 100 μm with the density of labeling rapidly diminishing with increasing distance from the ependymal surface. Dense immunoreactivity is present in perivascular spaces, along the pial surface of the ventral and lateral surface of the brain and in a few other areas, such as the supramammillary nuclei and the hippocampus. The apical ends of tanycytes in the organum vasculosum lamina terminalis and posterior periventricular nucleus of the hypothalamus are stained. Extremely sparse and faintly labeled neurons are evident in the subfornical organ, the anterior paraventricular hypothalamic nucleus, and other medial hypothalamic nuclei such as the dorsomedial, ventromedial, and arcuate nuclei, some of which extend processes towards the ependymal wall (Fig. 1b).

In the long-term survival groups, diffuse, bilateral labeling of the ventricular ependyma and, in most cases, the ventral pial surface of the brain continues to be present (Fig. 1c). For lateral ventricle injections, this zone of diffusion includes the septum, bed nucleus of the stria terminalis, nucleus accumbens and caudoputamen. For third ventricle injections, it includes the periventricular nucleus, anterior, dorsomedial, ventromedial, and arcuate hypothalamic nuclei. Dense retrograde neuronal labeling is present in numerous brain regions (see below). CTß also labels what appear to be small glia within fiber tracts and nerve roots close to the ventricles or the ventral surface of the brain.

2.2. *Injection sites*

Long-term injection sites can be divided into three groups: 1) injections without significant parenchymal deposition of tracer along the needle track but with dense ependymal labeling, indicating that tracer was deposited predominantly into CSF (Fig. 1c); 2) those with dense immunoreactivity along the needle track, indicating significant parenchymal deposition, as well as bilateral ependymal immunoreactivity (mixed cases); and 3) those with dense parenchymal immunoreactivity but without any ependymal labeling (Fig. 1d, control infusions). In the mixed injection cases of group 2, the pattern of retrograde labeling differs between brains but all of these brains still show a consistently labeled set of structures that is identical to that of group 1 brains. The essential findings of group 1 are confirmed by group 2. However, only data from group 1 are described below.

2.3. *Distribution of labeled neurons*

Although we do not rule out anterograde labeling, that is, uptake at the level of the cell body, it does not appear to be a primary source of neuronal label. CTß is an antero- and retrograde tracer but it is not taken up by neurons in the subependymal zone as expected from anterograde uptake (see parenchyma near the ventricles in Fig. 1c). Furthermore, anterogradely labeled CTß fibers are observed only in cases with extensive parenchymal involvement along the needle track (see corpus callosum in control case, Fig. 1d). Retrograde neuronal uptake sufficient to result in long-term labeling is likely to depend on multiple factors, including survival time, tracer concentration, affinity for various tissue components,