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Hypophysiotrophic TRH-Producing Neurons Identified by Combining Immunohistochemistry for Pro-TRH and Retrograde Tracing

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ABSTRACT

To determine hypophysiotrophic thyrotropin-releasing hormone (TRH)-producing neurons in the rat hypothalamus, we employed a combination of the immunohistochemistry for TRH prohormone (pro-TRH) and the retrograde tracing of neurons that project to the median eminence (ME) by injecting biotinylated wheat germ agglutinin (WGA) into the ME. In intact rats, immunoreactive pro-TRH-positive neurons occurred in the parvicellular paraventricular nucleus (parvi-PVN), basal part of the anterior and lateral hypothalamus, perifornical area and dorsomedial nucleus, especially accumulating in the parvi-PVN. Twenty-four hours after injection of the WGA into the middle portion of the ME, we found neurons that incorporated the lectin in the anterior periventricular area, the PVN, and the arcuate nucleus. When we examined serial sections consecutively stained with anti-WGA, anti-pro-TRH, and anti-WGA, most of the pro-TRH-labeled neurons in the medial parvi-PVN and a part of the neurons in the anterior periventricular area and in the anterior, lateral, and dorsal parvi-PVN appeared to incorporate WGA. These neurons may correspond with the hypophysiotrophic TRH-synthesizing neurons in the rat hypothalamus.

Key words: hypothalamus, localization, wheat germ agglutinin, rat

Since the antiserum for thyrotropin-releasing hormone (TRH) became available in immunohistochemistry (Hökfelt et al., '75a,b), many studies have demonstrated immunoreactive TRH neurons in the hypothalamus (Jackson and Reichlin, '77; Johansson and Hökfelt, '80; Brownstein et al., '82; Lechan and Jackson, '82; Nishiyama et al., '85). However, TRH was also found in other locations of the central nervous system, including the brainstem and spinal cord (Jackson and Lechan, '83; Lechan et al., '83; Ishikawa et al., '84). In these regions, TRH does not regulate thyrotrophs. Even in the hypothalamus, TRH immunoreactivity occurred in various locations, and much effort was concentrated on clarifying the locations of hypophysiotrophic TRH-containing neurons in various experimental ways (Martin and Reichlin, '72; Brownstein et al., '82; Lechan et al., '82; Palkovits et al., '82; Nishiyama et al., '85). However, substantial evidence is lacking to assess the neurons projecting to the median eminence, because of the methodological difficulty in determining the neurons that project to the median eminence to release the hormonal TRH. Recently, it was shown that an antiserum raised to a theoretical TRH progenitor sequence (pro-TRH-SH) stains neurons whose distribution corresponds to cells immunolabeled for TRH in the brain (Lechan and Jackson, '82; Jackson et al., '85; Lechan et al., '86; Merchenthaler et al., '89a). The serum revealed neurons in animals without colchicine pretreatment, which is commonly used to arrest axonal transport, and in animals fixed with conventional Bouin's fixative, in which anti-TRH serum did not reveal immunoreactive neurons. This conventional tissue preparation was also used in the immunohistochemical demonstration of wheat germ agglutinin (WGA) retrogradely transported from the nerve terminals in the median eminence to the cell bodies (Kawano and Daikoku, '87). Hence it became possible to identify hypophysiotrophic TRH neurons by applying a combination of immunohistochemistry for pro-TRH and retrograde tracing by injecting WGA to the median eminence.

MATERIALS AND METHODS

Preparation of anti-pro-TRH serum

The antiserum for pro-TRH was generated in rabbits against synthetic decapeptide, Cys-Lys-Arg-Gln-His-Pro-Gly-Lys-Arg-Cys (pro-TRH-SH; kindly supplied by Dr. F.

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