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THE PRESENCE OF TWO α-MSH POSITIVE CELL GROUPS IN RAT HYPOTHALAMUS

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ACTH-like and α-MSH-like immunoreactivity have been reported by several groups to be present in the arcuate nucleus of rat hypothalamus (cf. Watson and Akil, unpublished observations; Pelletier and Dube, 1977; Jacobowitz and O'Donnohue, 1977; Watson et al., 1978). Reports of these several groups are remarkably consistent in that a single major cell group was reported to be found in the arcuate nucleus, with a few cells scattered out laterally and with the fiber system projecting throughout hypothalamus, amygdala, nucleus accumbens, ventral-lateral septum, periventricular thalamus, periaqueductal grey and caudally to the level of the locus coeruleus. A few studies have demonstrated the occurence of ACTH-like immunoreactivity or α-MSH-like immunoreactivity with β-endorphin (β-END), β-lipotropin (β-LPH) or a 16K fragment of the endorphin-ACTH precursor within the same arcuate neurons (cf. Watson et al., 1978). From these reports it has become clear that immunoreactive portions of the 31K pro-opiocortin precursor (Mains et al., 1977) occur within the same cells in brain as they do in the anterior and intermediate lobe of pituitary. In the course of evaluating this hypothesis we have discovered a second set of α-MSH positive cells outside of the arcuate nucleus in rat hypothalamus. These cells are not positivie for β-END or β-LPH.

Four different α-MSH antisera were obtained from Dr. E. Weber, Ulm, Germany, and from Immunonuclear, Stillwater, Minnesota (No. 373, 378, 379). All of these antisera were blocked by 1 μM α-MSH, none were blocked by 1 μM ACTH 1-24, 17-39 or 1-39, β-END or β-LPH. β-END antisera and β-LPH antisera were a kind gift of Drs. R. Mains and B. Eipper, University of Colorado. These antisera were totally blockable with 1 μM of the appropriate peptide but were not blockable with ACTH or α-MSH. After heavy colchicine pretreatment (75 μg, i.c.v.) the rats were processed for immunohistochemistry as described elsewhere (Watson et al., 1978), and were sacrificed at 48 h. 4 μm frozen serial sections of rat were incubated in a sequential fashion with α-MSH and β-END or β-LPH. These very thin sections allow us to cut through many cells 3-4 times, permitting multiple stains of a given cell on adjacent sections.

As reported elsewhere, β-END cells and β-LPH cells are only seen in the arcuate nucleus (cf. Watson et al., 1978). In serial 4 μm sections α-MSH immunoreactivity was also detected in arcuate cells. By careful analysis of photographs of the entire arcuate area in these serial sections, it is possible to account for every α-MSH immunoreactive cell and every β-END and β-LPH cell, as containing all three peptides (Watson and Akil, unpublished observations). We now report that in an analysis of other areas of those same serial sections, α-MSH cell bodies were seen outside of the arcuate nucleus but were not positive for β-END or β-LPH. In the case of both sets of cell bodies, the histochemical demonstration could be consistently and completely blocked by 1 μM α-MSH. Fig. 1 demonstrates that α-MSH cells could be seen

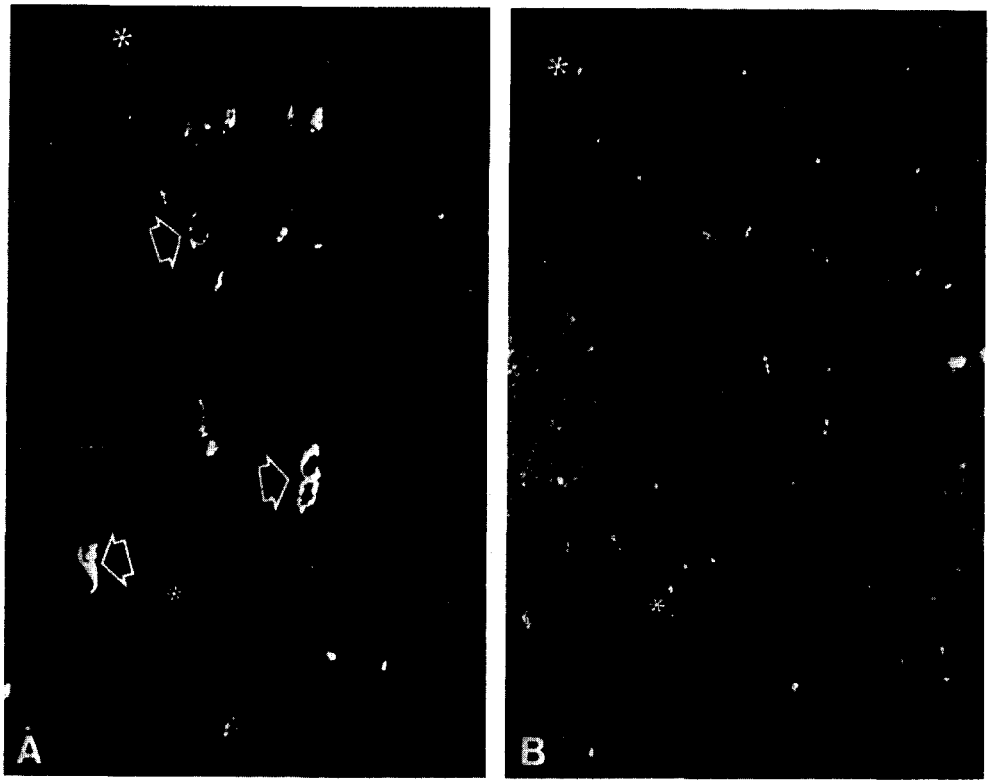

Fig. 1. Dorsal/third ventricular region in rat hypothalamus. Panel A shows α-MSH positive cells spreading loosely from the third ventricle laterally out into the zona incerta and toward the fornix. The single asterisk marks the third ventricle, the double asterisk marks a reference vessel (magnification ×240). Panel B is taken from a section 4 μm later, using β-END antiserum, demonstrating that no immunoreactivity was visible in the α-MSH cells seen in Panel A. In none of the cells outside of arcuate could β-END or β-LPH be detected (× 240).

near the top of the third ventricle. This cell group continues out into the zona incerta, down toward the fornix, on in a ventrolateral fashion toward the lateral hypothalamic sulcus and the insertion of the optic track. This rather large number of loosely packed cells was physically separate from the arcuate system and was noted to be consistently present in all rats studied. Using these same four antisera, fibers could be demonstrated in hippocampus and cortex. Such fibers have not been previously reported, using β-END or β-LPH antisera and are therefore hypothesized to be from the non-arcuate α-MSH cell bodies.

Thus, using heavy colchicine pretreatment, we have demonstrated a second group of α-MSH cell bodies which is apparently unrelated to the pro-opiocortin 31K arcuate system (Mains et al., 1977). It is of course possible that the arcuate and non-arcuate systems use a common biosynthetic system, but that the non-arcuate neurons process the intermediate portions of the pro-opiocortin very rapidly and are thus not visible within these neurons. In sum, it would appear that there are at least two neuronal systems involving the presence of α-MSH immunoreactivity, one in the arcuate β-END system and one in dorso-lateral hypothalamus. The presence of two such systems raises questions about the possibility of α-MSH receptors in brain, the anatomical connectivity of those systems and their relationship to behavior.

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