EFFECT OF β-ENDORPHIN ON CATECHOLAMINE LEVELS IN RAT HYPOTHALAMUS AND CEREBRAL CORTEX

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Endogenous opioid neuropeptides play an important role in the regulation of cell metabolism [2]. Neuropeptides and, in particular, β-endorphin, have been shown to possess a marked action on processes regulating the secretion and, possibly also, synthesis of pituitary hormones (prolactin, somatotrophic hormone — STH, ACTH), giving rise to stimulation of these functions of the pituitary gland [3, 5]. Neurochemical and neurophysiological aspects of the regulation of release of pituitary trophic hormones are closely linked with metabolism of biogenic monoamines [4, 7]. The first proof has now been obtained of the mediator and modulating role of opioid peptides, connected with the control of neurotransmitter secretion from nerve endings as a result of the formation of synapses with dopaminergic and noradrenergic nerve endings [6, 9]. It is not yet clear whether opioid peptides are true neurotransmitters, whether they play the role of neuromodulators of synaptic transmission, or whether they are neurohormones.

The aim of this investigation was to study the effect of β-endorphin on catecholamine concentrations in the hypothalamus and cerebral cortex, as a contribution to the explanation of the mechanism of action of this peptide on certain pituitary trophic functions.

EXPERIMENTAL METHODS

Experiments were carried out on male rats. β-Endorphin (from ICN Pharmaceuticals, Inc., USA) was injected intravenously (1 ng/kg body weight). The animals were decapitated 5, 20, and 60 min later and the brain removed for investigation. This dose was used on the basis of the results of an investigation [10] into the effect of exogenous β-endorphin on certain physiological parameters in rats, and of our own results relating to the action of this same dose of β-endorphin on blood ACTH and prolactin levels and pituitary STH level in rats [3]. The material was processed at 2-4°C. Weighed samples of tissue from the cerebral cortex and hypothalamus were homogenized in freshly prepared cold 0.4 N perchloric acid solution (10 ml to 1 g of tissue), containing 5 mM reduced glutathione, then centrifuged at 2500g (10 min, 2-4°C). The supernatant was kept at −20°C in tightly closed test tubes. The concentrations of dopamine (DA), noradrenalin (NA), and adrenalin were determined by a radioenzymic method using standard kits (from Upjohn Diagnostics, USA). A Mark 3 scintillation system (from Tracor Europa) was used for radiometric investigation of the samples.

RESULTS

β-Endorphin affects the catecholamine concentration in the rat brain, and the intensity of the effect depends on the topography of the brain structure and the time after injection of the peptide. No change in catecholamine levels either in the hypothalamus or in the cerebral cortex was observed 5 min after injection of β-endorphin. However, 20 min after injection of the peptide a marked fall of the DA, NA, and adrenalin levels were observed in the hypothalamus (Table 1). After 60 min the catecholamine level in the hypothalamus was higher than in the control, but the differences were not significant. In the cerebral cortex, 20 and

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TABLE 1. Concentrations (in pg/100 mg) of DA (I), NA (II), and Adrenalin (III) in Rat Hypothalamus and Cerebral Cortex at Different Times after Injection of β-Endorphin (M ± m; n = 7)

Test object	Time after injection of β-endorphin, min					
	0			5		
	I	II	III	I	II	III
Hypothalamus	544,0±59,0	919,0±175,3	1085,0±249,7	444,0±270,0	986,0±234,7	874,0±235,3
Cerebral cortex	302,0±94,6	1210,0±271,0	1279,0±261,4	326,0±201,8	927,0±181,6	831,0±189,3

Test object	Time after injection of β-endorphin, min					
	20			60		
	I	II	III	I	II	III
Hypothalamus	147,0±34,4*	224,0±45,0*	531,0±108,9*	334,0±93,9	514,0±107,7	744,0±144,2
Cerebral cortex	238,0±92,0	632,0±243,8	681,0±219,6	275,0±62,0	713,0±173,8	812,0±171,2

Note. *P < 0.05 compared with initial level.

60 min after injection of β-endorphin, no significant changes were found in the catecholamine levels, although there was a tendancy for them to fall (Table 1).

The results of these experiments thus indicate that β-endorphin has a marked effect on brain catecholamine levels, mainly in the hypothalamus, the structures of which are involved in regulation of pituitary trophic functions [1, 7]. Evidence of the influence of opioid peptides, polypeptide P, and neurotensin on the concentration and release of catecholamine in the brain (corpus striatum, nucleus accumbens) also is described in publications by other workers [12, 15].

When the possible mechanisms of action of opioid peptides on the brain catecholamine levels are analyzed, a modulatory role can be postulated for endogenous opioids regulating neurotransmitter (catecholamine) release. For instance, intraventricular injection of β-endorphin (2.9 nanomoles) reduces the acetylcholine turnover in the cortex, hippocampus, nucleus accumbens, and globus pallidus and increases it in the hypothalamus and median eminence [11]. Opiate neuropeptides stimulate induced NA secretion from sections of the cortex and serotonin secretion from the brain stem and hypothalamus [13]. It has been shown [14] that β-endorphin increases the blood catecholamine concentrations, chiefly on account of the adrenals, but under these circumstances marked stimulation of release of DA, NA, and adrenalin is observed from central adrenergic nerve endings. The above hypotheses are confirmed by data on the regional distribution of β-endorphin in brain structures which point to the existence of synaptic contacts between neurons containing β-endorphin and terminals of dopaminergic neurons [14]. Some workers [8], however, attempting to explain the mechanism of action of opiate neuropeptides at the catecholamine level, have suggested that peptides modify activity of enzymes catalyzing mediator biosynthesis and, in particular, tyrosine hydroxylase, the key enzyme for catecholamine synthesis.

It is possible that opioids affect catecholamine release by altering the pathway of monoamine biosynthesis [8]. Our data on the effect of β-endorphin on the catecholamine concentration in the rat brain also suggest possible changes in tyrosine hydroxylase activity, evidence of which is given by the proportional decrease in the DA and NA concentrations 20 min after injection of the peptide.

Thus β-endorphin causes changes in catecholamine metabolism, expressed as a fall in their concentration in brain structures such as the hypothalamus and cerebral cortex as early as 20 min after injection of the neuropeptide. At this same time, as the writers showed previously [3], after injection of β-endorphin the maximal increase is observed in the blood levels of ACTH, prolactin, and STH in rats. Participation of catecholamines in the regulation of pituitary trophic functions, which are depressed under the influence of DA and NA, and the opposite effect when they are deficient, can now be regarded as proven. In a study of the distribution of adrenergic systems in the hypothalamus it has been clearly shown that the highest DA and NA concentrations are found in those hypothalamic structures that participate in

the regulation of certain pituitary trophic functions and, in particular, corticotrophic, lactotrophic, somatotrophic, and thyrotrophic [1]. Accordingly, one of the mechanisms lying at the basis of the stimulating action of β-endorphin on secretion of ACTH, prolactin, and STH is evidently a fall in the concentration of catecholamines and, in particular, DA and NA, in the hypothalamus. This leads to activation of specific hypothalamic factors (and somatotrophin releasing factors) and to release of the pituitary trophic hormones.

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ACTION OF CYTOCHALASIN D ON DNA SYNTHESIS IN CELLS IN CULTURE

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To explain the role of changes in the cytoskeleton and, in particular, cortical microfilaments in the regulation of cell multiplication, the effect of cytochalasin B (CCB), which destroys the system of actin microfilaments, on proliferation of cells in culture has been studied. It has been shown that CCB inhibits DNA synthesis in normal cells [2, 4, 6]. However, it has not been explained whether this effect is the result of changes in the cytoskeleton, for two other possible explanations have not been ruled out. We know that when cells are incubated in the presence of CCB, the last stage of mitosis (cytokinesis) is disturbed [1], and as a result of this part of the culture becomes binuclear. Some workers explain the inhibition of DNA synthesis during culture of cells in CCB by the direct inhibitory effect of the two nuclei on each other, the so-called "effect of restriction of polynuclearity of normal cells" [5]. Another problem not yet solved is whether inhibition of DNA synthesis is the result of a side effect of CCB — blocking of glucose transport into the cell [3].

In the present investigation, to solve the problem of the effect of changes in the actin cytoskeleton on DNA replication during the action of cytochalasins, the effect of long-term incubation of normal cells with cytochalasin D (CCD), which selectively destroys the microfilament system but does not affect transport of sugars, when investigated. Incorporation of labeled thymidine into mononuclear and binuclear cells in the presence of CCD and after its removal by rinsing also was studied separately.

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