POSSIBLE ROLE OF SOMATOTROPHIC HORMONE IN GLIAL CELL FUNCTION REGULATION IN THE CNS

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It is generally considered that the principal biological function of somatotrophic hormone (STH) is to stimulate the somatic growth of animals and man. Nevertheless its role in regulation of the functional state of cells of the CNS under normal and pathological conditions has not hitherto been examined. However, the discovery of STH and receptors for somatomedins (hypothetical mediators of the growth effect of STH) in different parts of the mammalian brain [8, 12, 15], and the discovery of a protein factor with molecular weight of 30 kilodaltons, stimulating growth of glial cells in pituitary extracts [5, 11], are evidence of the need for research into the role of STH in the regulation of cell functions in the CNS and, in particular, in proliferation of glial cells or regulation of the growth of their processes. Since catecholamines (CA) and cyclic 3',5'-adenosine monophosphate (cAMP), unlike STH, inhibits the growth of cells, including glial cells [6, 10], it can be postulated that one way by which STH affects growth of glial cells is through modulation of the reception or inactivation of CA in these cells.

Accordingly in the investigation described below the effect of STH and of its fragment preserving growth-stimulating activity was studied, on the one hand, on activity of monoamine oxidase (MAO), a key enzyme of CA metabolism, and on the other hand, on selective binding of CA by α- and β-adrenoreceptors of glial cells. For comparison, their effect on binding of prostaglandin E_1 (PGE_1) with the membranes of these same cells was studied.

EXPERIMENTAL METHOD

[^{3}H]Dihydroalprenolol ([^{3}H]-DHA, 60 Ci/mmole), [^{3}H]dihydroergocryptine ([^{3}H]-DHE, 17.5 Ci/mmole), and [^{3}H]-PGE_1 (50 Ci/mmole) were used; all were from Amersham Corporation, England; 4-alprenolol and phentolamine were from Sigma, West Germany; STH was isolated from the sei whale pituitary; the 31-membered peptide used corresponded to regions 77-107 of the amino-acid sequence of STH. The method of obtaining and characterizing STH and its fragments were described in [3]. Preparations of STH and its fragments were homogeneous, as shown by the results of N-terminal amino-acid analysis.

A fraction rich in glial cells was obtained from rat cerebral cortex in a weight of 60-70 g by the method [4] modified by the writers. Minced brain tissue was passed through nylon sieves with pore diameters of 1000, 500, 250, 150 and 90 μ in a solution containing 7.5% polyvinylpyrrolidone, 10 mM EDTA, and 1% bovine serum albumin, and centrifuged at 250g for 15 min. The residue, containing glial cells and neuron bodies, was treated with 20% Ficoll and the cell suspension was again passed through sieves with pore diameters of 500, 250, 150, and 90 μ. The cell suspension was then added to the middle (between 15 and 30%) of a continuous Ficoll density gradient (10, 15, 20, 30, and 47%) and centrifuged at 54,000g for 120 min. Glial cells floating during centrifugation from the 20% Ficoll zone into the 15% Ficoll zone (the neurons, on the other hand, sank down to the 47% Ficoll zone) were removed, 0.32M sucrose was added, and the suspension was centrifuged at 600g for 60 min. Of the fractions thus obtained, as phase-contrast microscopy showed, glial cells accounted for 80-85% of the total. After washing 3 times, the glial cells were homogenized in 10 mM Tris-HCl, pH 7.4, frozen, and kept at −30°C.

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TABLE 1. Effect of STH and Its Fragments on MAO Activity (in absolute units) in Rat Brain Glial Cell Homogenates (M ± m, n = 7)

Substance tested	Concentration, M	MAO activity
Control	—	5,6±0,8 (100)
STH	10^{-6}	5,4±0,3 (96,4)
STH fragment	10^{-6}	5,7±0,4 (101,8)
Transamine	10^{-8}	0,6±0,1 (10,7)*

Legend. Here and in Table 2, percentage of control given in parentheses. *P < 0.01.

TABLE 2. Effect of STH and Its Fragment on Specific Binding of [^{3}H]-DHE, [^{3}H]-DHA, and [^{3}H]-PGE_1 (in pmoles/mg protein) in Rat Brain Glial Cell Homogenates (M ± m, n = 7-9)

Substance tested	Concentration, M	[^{3}H]·DHE	[^{3}H]·DHA	[^{3}H] PGE_1
Control	—	2,42±0,08 (100)	0,60±0,03 (100)	0,23±0,01 (100)
STH	10^{-6}	2,18±0,08 (90,1)	0,38±0,01 (63,3)	0,22±0,01 (95,7)
	$5\cdot10^{-9}$	4,30±0,10 (177,7)	0,42±0,02 (70,0)	0,23±0,02 (100)
STH fragment	10^{-6}	2,16±0,07 (89,3)	0,35±0,01 (58,3)	0,21±0,03 (91,3)
	$5\cdot10^{-9}$	4,38±0,10 (181)	0,40±0,01 (66,7)	0,23±0,01 (100)

Legend. For [^{3}H]-PGE_1 difference is never statistically significant; in all other cases P < 0.01.

To determine MAO activity or to investigate ligand–receptor interaction, 50-µl samples of glial cell homogenate were obtained (0.6-0.8 µg protein) and added to incubation tubes with a final volume of 300 µl. Binding of [^{3}H]-DHA with β-adrenoreceptors was determined by the method in [7], and binding of [^{3}H]-DHE with α-adrenoreceptors by the method in [14], and binding of [^{3}H]-PGE_1 by the method in [9]. The [^{3}H]-DHA and [^{3}H]-DHE were incubated for 15 min with glial cell homogenate at 25°C at pH 8.0, and the [^{3}H]-PGE_1 was incubated at 37°C and pH 7.4. Labeled ligands, bound with glial cell membranes, were separated from unbound by passing the incubation mixture through GF/C glass filters (Amersham Corporation) and washed with 15 ml of cold Tris-HCl, pH 7.4. Specific binding of the labeled ligands by the corresponding glial cell receptors was judged from the difference between total and nonspecific binding. The latter was determined by adding a 100-fold excess of unlabeled specific ligands to the incubation mixtures: alprenolol for β-adrenoreceptors, phentolamine for α-adrenoreceptors, and PGE_1 to detect prostaglandin receptors. The radioactivity of the samples was measured on a Mark III counter (USA). To measure MAO activity, 4-nitrophenylethylamine was used as the substrate, by the method in [2]. Activity of the enzyme was measured in the presence of 1% Triton X-100 in thermostatically controlled (37°C) microcuvettes of a Specol (West Germany) spectrophotocolorimeter for 10 min. MAO activity was judged from the increase in optical density of the medium at 450 nm as the result of oxidative deamination of the 4-nitrophenylethylamine. Protein was determined by the method in [13]. The experimental data were subjected to statistical analysis on HP-9815A computer (USA). The significance of differences was estimated by Student's t test.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of STH and its fragments, in concentrations of 10^{-6} and 10^{-9} M, on MAO activity was studied. Table 1 shows that STH and its fragments in a concentration of 10^{-6} M had virtually no effect on basal activity of the enzyme, whereas transamine, a specific inhibitor of MAO activity, suppressed it almost completely. STH and its fragments in a concentration of 10^{-9} M also were ineffective. Consequently, STH and its

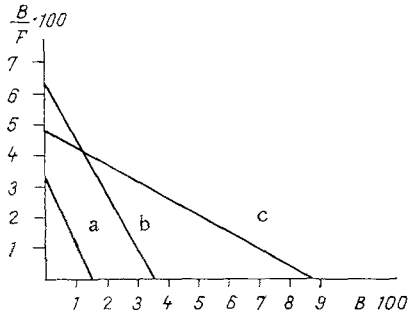

Fig. 1. Scatchard plot analysis of binding of [^{3}H]-PGE$_1$ (a), [^{3}H]-DHA (b), and [^{3}H]-DHE (c) with glial cell membranes. B) Number of specifically bound ligands (in fmoles/mg protein); B/F) ratio of bound to free ligands.

fragments were unable to reduce the CA content in the CNS on account of induction of MAO activity and, naturally, they could not block the inhibitory effect of CA on growth of glial cells in the same way [10].

To detect other possible ways whereby STH may influence the state of glial cell function, its effect on binding of CA with α- and β-adrenoreceptors of glial cells was studied. For comparison, the effect of STH and its fragments on specific binding of [^{3}H]-PGE$_1$ also was studied. To create optimal conditions for detecting the effects of STH and its fragment on ligand–receptor interaction in glial cells, reception of each ligand was plotted between Scatchard coordinates (Fig. 1). Calculations showed that for [^{3}H]-DHE K_d = 8.2 nM and B_{max} = 860 fmoles/mg protein; for [^{3}H]-DHA K_d = 4.1 nM and B_{max} = 352 fmoles/mg protein; for [^{3}H]-PGE$_1$ K_d = 1.8 nM and B_{max} = 250 fmoles/mg protein; for each ligand studied Hill's coefficient was 1. Investigation of the effect of STH and its fragment on ligand–receptor interaction showed (Table 2) that STH and its fragment in a concentration of 10^{-6} M significantly reduced specific binding of [^{3}H]-DHA but inhibited specific binding of [^{3}H]-DHE with β- and α-adrenergic receptors of glial cells respectively only weakly. With a decrease in the concentration of STH and its fragment to $5 \cdot 10^{-9}$ M the inhibitory effect on binding of [^{3}H]-DHA was preserved. In the same dose, conversely, they both considerably increased specific binding of [^{3}H]-DHE with glial cell α-adrenoreceptors.

STH and its fragment, in the concentrations tested, did not affect specific binding of [^{3}H]-PGE$_1$ with prostaglandin receptors of the glial cells, which points to definite specificity of their action on adrenergic reception. An essential difference in the effects of STH and its fragment is that they only partially blocked or activated α- and β-adrenoreception. This suggests that STH and its related peptide can selectively influence only certain subpopulations of adrenoreceptors (β_1 or β_2, α_1 or α_2). The ability of STH and its fragment to modify the direction of the effect of CA on individual adrenoreceptor subpopulations in glial cells, and so to selectively induce particular intracellular biochemical reactions is in agreement with the writers' previous observations on their effect on basal adenylate cyclase and phosphodiesterase activity in glial cells. STH has only a weak effect on basal adenylate cyclase activity, and its fragment has no action on this enzyme, but both activate phosphodiesterase, the fragment, indeed, doing so particularly strongly [1].

It can be tentatively suggested that STH and its fragment may modulate the functional state of glial cells by changing the direction of action of CA on subpopulations of adrenergic receptors, thereby facilitating induction of Ca-dependent intracellular reactions (through α-adrenoreceptors), coupled with lowering the intracellular cAMP level. The fall in the cAMP level in the glial cells may, in turn, act as inducing factor for proliferation and differentiation of these cells. The inhibitory action of cAMP on glial cell proliferation has been demonstrated experimentally [10]: Addition of dibutyryl-cAMP or theophylene or activation of adenylate cyclase by the β-adrenomimetic isoprenaline sharply inhibits DNA biosynthesis in glioma C6 cells in culture.

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CARDIAC cAMP AND cGMP DYNAMICS IN RATS WITH TRANSIENT CORONARY INSUFFICIENCY

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The principles and role of changes in the content of cyclic nucleotides (CN) — cAMP and cGMP — in the myocardium during ischemia and hypoxia of that structure have been studied intensively in recent years [1, 11-15]. However, no publications could be found relating to the study of the dynamics of CN concentrations in the heart in coronary ischemia and during the period of subsequent restoration of the coronary blood flow. Yet such information is of great interest not only to experimental, but also to clinical cardiology, for in most cases ischemic heart disease is manifested as transient coronary insufficiency (TCI), characterized by resumption of coronary perfusion after a period of myocardial ischemia (MI) of a certain duration. TCI in man occurs in three principal forms: angina, an intermediate coronary syndrome, and states after surgical (aortocoronary bypass) or drug-induced (thrombolysis) myocardial revascularization in the acute stage of infarction [2, 6, 10]. It will be recalled that disturbances of regulation of the contractile function and rhythm of the heart in animals and in man are observed both in the period of MI and in the early stage of postischemic reperfusion (PIR) [2, 3, 6].

Previously the writers discovered that the dynamics of heart function in TCI depends essentially not only on the degree and scale of myocardial damage, but also on the character of extra- and intracardial regulatory influences on it [5, 6, 8]. At the cardiomyocyte level the realization of these controlling influences is effected largely through cAMP and cGMP. This applies most of all to extracardial adrenergic and cholinergic mechanisms of regulation, whose activity regularly changes in TCI [5, 8].

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