Stimulation of Cyclic AMP Accumulation in the Rat Anterior Pituitary in vitro by Analogs of Luteinizing Hormone-Releasing Hormone

Anterior pituitary cyclic AMP accumulation appears to play a role in mediating the action of luteinizing hormone-releasing hormone (LH-RH, pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) in the gland. Parallel stimulation of the cyclic AMP and release of LH, and also follicle-stimulating hormone, has been observed in the presence of LH-RH in vitro[1-3]. Also, LH release is increased by N^6-$O^{2'}$-dibutyryl cyclic AMP and theophylline, a cyclic-nucleotide phosphodiesterase inhibitor[4].

Synthetic analogs of LH-RH exhibit the above activities of the LH-RH with varying potencies[2,3]. In order to further define the structure-activity relationship, in the present study various additional LH-RH analogs have been examined for their ability to cause stimulation of the cyclic AMP accumulation.

Materials and methods: The method employed was essentially as that described previously[1]. Hemipituitaries from male adult Sprague-Dawley rats (Canadian Breeding Laboratories) were employed. 3 hemipituitaries were used in each group and there were 8 groups in each determination. The test compounds were dissolved in a vehicle of 0.1 *N* acetic acid and 10% ethanol. The appropriate aliquot was removed, evaporated to dryness and subsequently redissolved with the medium. The pre-incubation period was 1 h followed by an assay incubation period of 3 h. All glassware utilized was coated with 1% bovine serum albumin.

The cyclic AMP was extracted from the tissues with 5% trichloroacetic acid and measured by the receptor-binding assay of GILMAN[5] utilizing 10 µg of protein of the inhibitor and 1 µg of receptor preparation (P-5511, Sigma Chemical Co.). [8-^{3}H] Cyclic AMP (Schwartz-Mann Co; 24–28 Ci/mmole was employed at a final concentration of 40 n*M*. Unlabelled cyclic AMP was obtained from Calbiochem Co. Assays were performed in triplicates. After filtration, the filters were dried and 10 ml toluene phosphor [0.4% 2,5-diphenyloxazole and 0.005% 1,4-*bis* (5-phenyloxazole-2-yl)benzene] employed for scintillation counting. The LH-RH analogs were prepared by Drs. H. IMMER, V. NELSON and K. SESTANJ, Ayerst Research Laboratories.

Results. LH-RH at 0.5 ng/ml caused a 3-fold increase in the cyclic AMP accumulation; about a 1.5-fold rise occurred at 0.1 ng/ml (Table). Lys^8-LH-RH at 1 ng/ml did not affect the cyclic AMP level while at 5 ng/ml the analog increased the level 2-fold; similar activities were obtained with phe^5-LH-RH. These levels of activities were obtained with the ala^4-LH-RH at 4 and 20 ng/ml, respectively, and ala^5-LH-RH at 10 and 50 ng/ml, respectively. No appreciable stimulation occurred with des-pro^9-LH-RH at 50 ng/ml.

Theophylline (10^{-2} *M*), an inhibitor of cyclic nucleotide phosphodiesterase, caused a 6-fold increase in the cyclic AMP accumulation (Figure). In the presence of the appropriate concentration of each of the analogs as determined above, the increases in cyclic AMP were observed even in the presence of theophylline. Thus, the LH-RH analogs appear to be acting by stimulating the adenyl cyclase rather than inhibiting the phosphodiesterase.

Effects of LH-RH and analogs on cyclic AMP accumulation in rat anterior pituitary

Peptide	Concentration (ng/ml)	Cyclic AMP (% of control)
LH-RH	0.1	139 ± 27
	0.5	297 ± 79
Lys^8-LH-RH	1.0	120 ± 23
	5.0	223 ± 38
Phe^5-LH-RH	1.0	113 ± 9
	5.0	236 ± 21
Ala^4-LH-RH	4	120 ± 17
	20	255 ± 26
	5	157 ± 17
	25	261 ± 33
Ala^5-LH-RH	10	128 ± 12
	50	223 ± 20
Des-Pro^9-LH-RH	50	134 ± 14

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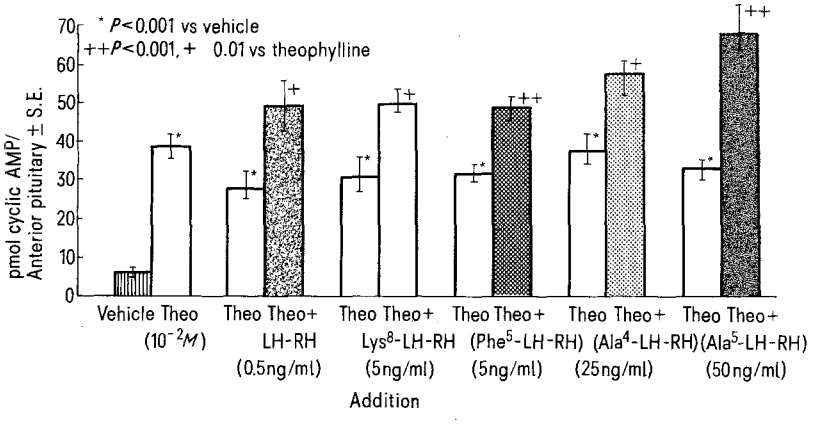

Effect of LH-RH and analogues and theophylline on cyclic AMP accumulation in rat anterior pituitary.

Discussion. The present findings show the relative potencies of various LH-RH analogs, i.e. the lys[8]-, phe[5]-, ala[4]-, ala[5]- and des-pro[9]-LH-RH, for causing an increase in the cyclic AMP accumulation in the anterior pituitary in vitro thus yielding a more definitive structure-activity relationship with regard to this activity. In other in vitro studies, different LH-RH analogs have also been shown to increase the cyclic AMP levels with a close parallelism being observed between the changes in the cyclic AMP accumulation and the LH release[3].

In the present studies, the highest activity for causing cyclic AMP accumulation was obtained with the lys[8]-LH-RH and the phe[5]-LH-RH, the activity being relatively high, i.e. about $^1/_{10}$ that of LH-RH. Thus these findings obtained when there was a replacement of the lysine for the arginine indicate the importance for the group in position 8 being basic in nature. This importance is also indicated by the existence of a similar relative relationship between these analogs with respect to LH release in vitro[6]. The present results obtained with the phe[5]-LH-RH demonstrate that the hydroxyl group of the tyrosine is relatively not critical. This observation is in accord with findings of others[3] with regard to cyclic AMP accumulation in a similar in vitro system and to LH release in vivo[7]. Further, high activities were obtained with the *O*-methyl-tyr[5]-LH-RH[7].

The hydroxy group of the serine can also be replaced by a hydrogen atom although the ala[4]-LH-RH is less potent than the phe[5]-LH-RH. These findings are consistent with the observations that the hydroxy group of the serine at position 4 is not essential for LH release in vitro[6] or in vivo[7].

That further reduction in activity results when the aromatic *p*-hydroxy-phenyl group of the tyrosine is replaced by a hydrogen atom and shortening of the chain length results in a loss of the activity are indicated by the findings with the ala[5]-LH-RH and the des-pro[9]-LH-RH, respectively. Consistent with the latter observation are the findings that little, or no, LH releasing activity in vivo is exhibited by smaller fragments of the LH-RH, e.g. the N-terminal tripeptides and tetrapeptides, and also the C-terminal nonapeptide and octapeptide[8] or the des-arg-LH-RH[9]. Also, the des-(pyro)glu[1]-LH-RH[2], des-(pyro)-glu[1]-his[2]-LH-RH[2], decapeptide-OH and tripeptide pyro(glu)-ser-val-NH_2[3] do not exhibit any appreciable effect on the cyclic accumulation or LH release in vivo. However, levels of these activities greater than those of the LH-RH are observed with the des-gly[10]-LH-RH ethylamide; removal of the histidyl residue in position 2 from this analog essentially abolishes the activities[2].

Other alterations have demonstrated that the leucyl residue at position 7 can be replaced by an isoleucine as the latter exhibits activities on the cyclic AMP accumulation and LH release in vitro similar to those of LH-RH[3]. Substitution of a phenylalanyl residue for the tryptophan at position 3 results in an analog which is not appreciably effective[3]. Also, reversing the position of the proline and arginine to yield the pro[8], arg[9]-LH-RH abolishes these activities[2].

The present findings as well as those cited are consistent with the existence of a role of cyclic AMP in the mediation of the action of the LH-RH and analogs.

Résumé. Pour déterminer in vitro la relation structure-activité dans l'hypophyse antérieure du rat, on démontre que différents analogues de l'hormone LH-RH («luteinizing hormone-releasing hormone») ont stimulé l'accumulation de l'AMP cyclique. Il semble que ces analogues exercent leur activité en stimulant l'enzyme adényle cyclase plutôt que par l'inhibition de l'enzyme phosphodiestérase.

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Regional Distribution of Adenine Nucleotides, Glycogen, Glucose and Lactate in the Adult Rat Brain

It is well known that various parts of the brain are at different levels of biochemical organization, depending primarily of the distinct phylogenetic ages. During the evolutionary development of the brain, replacement of the more anaerobic by aerobic metabolism takes place[1], and at the same time the processes of biological oxidation and oxidative phosphorylation become more intensive in the younger parts of the brain[1]. For the better understanding of the very complex functions of the brain, it is of particular importance to find out the energy status and the extent of the glycolysis in the energy production in the brain regions that are at different evolutionary levels, because of the very close relation between the brain metabolism and neuronal activity[2]. The aim of this work was to determine the levels of adenine nucleotides (ATP, ADP and AMP), glycogen, glucose and lactate in the three phylogenetically differently aged parts of the rat brain: frontal lobes, cerebellar hemispheres and medulla oblongata.

Materials and methods. The investigations were carried out on adult male Wistar rats. Nonanesthetized animals were killed by decapitation, and the heads were immediately immersed in liquid nitrogen. Brain parts were removed in the cold (0°–4 °C), and all subsequent procedures of extraction and centrifugation were done under the same cold conditions, according to FOLBERGOVA et al.[2]. Adenine nucleotides, glucose and lactate were assayed in the neutralized perchloric acid extracts as

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