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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[10] The author wishes to acknowledge the technical assistance of Miss GAIL CAREW-GIBSON.

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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Table I. Regional distribution of adenine nucleotides in adult rat brain

	Brain part		
	Frontal lobes	Cerebellar hemispheres	Medulla oblongata
ATP	1.76 ± 0.12 [a, b]	2.22 ± 0.15 [b]	1.52 ± 0.14
ADP	0.71 ± 0.08	0.88 ± 0.10 [b]	0.57 ± 0.05
AMP	0.26 ± 0.06	0.27 ± 0.05	0.31 ± 0.04
Total adenine nucleotides	2.73 ± 0.21 [a]	3.39 ± 0.21 [b]	2.50 ± 0.15
ATP/ADP ratio	2.59 ± 0.25	2.74 ± 0.33	2.67 ± 0.46
Energy charge potential [c]	0.78	0.79	0.76

Values (mM/kg wet tissue) represent the mean (M) ± S.E.M. for 6 animals. Student's t-test was performed for comparisons. [a] $P < 0.05$ relative to cerebellar hemispheres. [b] $P < 0.05$ relative to medulla oblongata. [c] Energy charge potential (ECP) was calculated according to formula[12]: ECP = (ATP + 1/2 ADP) / (ATP + ADP + AMP).

described elsewhere[3]. Glycogen was extracted and estimated as previously reported[4].

Results and discussion. The values for adenine nucleotides, as well as some calculated parameters, are shown in Table I. It is obvious that there are statistically significant ($p < 0.05$) regional differences in the distribution of ATP, ADP and total adenine nucleotides in rat brain. The highest values for ATP, ADP and total adenine nucleotides were found in cerebellar hemispheres (Table I), compared with the frontal lobes and medulla oblongata. This could be due to the more intensive oxidative metabolism in this part of brain. This supports the finding of DIXON and MEYER[5] that the respiration in the cerebellar cortex is about 50% greater than in the cerebral cortex. It was stated elsewhere[6] that the cerebellum has high energy status, and it seems that all these facts may be due to the very intensive neuronal activity of the Purkinje cells[7].

The values for the ATP/ADP ratio and energy charge potential (Table I) indicate that our findings probably reflect the in vivo state. We did not find any difference in AMP distribution (Table I). Since there is a difference in the distribution of the total adenine nucleotides, such finding for AMP could be the consequence of the a) unequal rate of the AMP transformation into inosine and/or b) distinct activities of the adenylate kinase isozyme III, which has been shown to play a very important role in the processes of the oxidative phosphorylation[8].

Distributions of glycogen, glucose and lactate are shown on Table II. The greatest glycogen content in medulla oblongata and the least in frontal lobes (Table II) conforms with the previously published data[4]. It seems natural that the value for glycogen is the greatest in the evolutionary older part of the brain, because of its dependence to a great extent on the anaerobic carbohydrate metabolism[1], assuming that glycogen is one of the most important brain energy reserves[9]. It is well known, too, that when anoxia arises the utilization of glycogen does not take part before glucose is almost completely exhausted[9–11]. The greater glycogen content in medulla oblongata probably enables vital centres to survive anoxia for a longer period, because glycogen is the main source of glucose under this condition; on the other hand, it has been shown that glucose appears to be the limiting factor for the processes of neurotransmition during anoxia[11].

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Table II. Regional distribution of glycogen, glucose and lactate in adult rat brain

	Brain part		
	Frontal lobes	Cerebellar hemispheres	Medulla oblongata
Glycogen	54.1 ± 1.3 [a, b]	90.0 ± 2.3 [b]	102.4 ± 4.4
Glucose	0.37 ± 0.03 [a, b]	0.52 ± 0.07 [b]	0.71 ± 0.03
Lactate	5.31 ± 0.28 [a, b]	6.31 ± 0.20 [b]	7.22 ± 0.31

The amount of glycogen is expressed in mg/100 g of tissue; the amount of glucose and lactate are given in mM/kg wet tissue. The numbers indicate the mean value (M) ± S.E.M. for 6 animals. Student's t-test was performed for comparisons. [a] $P < 0.05$ relative to cerebellar hemispheres. [b] $P < 0.05$ relative to medulla oblongata.

Distribution of glucose and lactate in the brain parts we investigated is simmilar to that of glycogen (Table II). These findings suggest that there is more intensive glycolysis in medulla oblongata, compared with the other parts of brain.

Résumé. Le cervelet contient des quantités plus grandes d'ATP, d'ADP et de nucléotides totaux que le lobe frontal du cerveau ou la moelle cervicale. Ces différences ne sont pas trouvées avec l'AMP. Le contenu en glycogène, en glucose et en lactate est plus grand dans la moelle cervicale que dans le cervelet et dans le lobe frontal.

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[13] We wish to thank Dr. H. REHKÄMPER from Boehringer Mannheim GmbH, who kindly supplied us with all the enzymes, substrates and cofactors.

Methyl Mercury Effect on Rat Liver Mitochondrial Deshydrogenases

Much attention has been paid to mercury and its organic derivates as they accumulate in the food chain[1]. Although there are many observations on biochemical alterations induced by organomercury compounds[2], the way methyl mercury exerts its toxicity on target organs is still subject to controversial interpretation: methyl-mercury reacts with sulfhydryl groups present at the active sites of several enzymes as other mercurials do[3,4]. According to SEGALL and WOOD[5], it can also catalyze the hydrolysis of a group of phospholipids.

Liver tissue has been selected because it accumulates methyl mercury and participates in the entero-hepatic reabsorption of the compound. Furthermore, recent ultrastructural foetal liver electron microscopy has shown mitochondrial swelling after low exposures which did not produce maternal neurological symptoms[6]. Therefore, besides its already recognized detoxification role, liver has a degenerative response to methyl mercury.

We have investigated the effect of methyl mercury on isolated inner membranes of rat liver mitochondria and its inhibitory action on several enzymes of the oxydative chain. Work has been focused on 3 hydroxybutyrate deshydrogenase, which requires lecithin as a cofactor[7] and has thiol residues in the active site[8]. A protective effect was obtained after a pre-incubation with the phospholipid and a sulphydryl reagent.

Material and methods. Male Wistar rats (180–200 g) were sacrificed. Livers were removed and mitochondria were extracted in 0.25 *M* sucrose. Inner membrane matrix preparation was obtained by digitonin treatment and disrupted by sonication according to the method of LEVY et al.[9]. Respiration rate and enzymatic activities: succinate deshydrogenase, 3-hydroxybutyrate deshydrogenase, cytochrome oxydase were assayed according to several methods detailed in a previous publication[10]. The protein content of the enzymatic preparation was determined by the method of LOWRY[11].

Liposomes of egg lecithin were prepared according to the method of SINGLETON et al.[12]: 2 mercaptoethanol was diluted to 0.5 *M* in phosphate buffer 0.2 *M* pH 7.4. Methyl mercuric chloride solutions in aq. 5% (v/v) ethanol were prepared just before use. The amount of ethanol added with the inhibitor has no significant effect on the activities assayed. Inner membranes were incubated for 5 min with the inhibitor before measuring the enzymatic activity.

Results and discussion. Some results of the inhibitory effect of methyl mercury on oxidase and deshydrogenase activities are shown in the Figure. A decrease in oxygen consumption is observed on both substrates. However, methyl mercury concentrations should not be considered as absolute values, since manometric operations require an incubation temperature (30 °C) at which methyl mercury vaporization is significant. Enzymatic assays show that 50% inhibition of 3 hydroxybutyrate activity is

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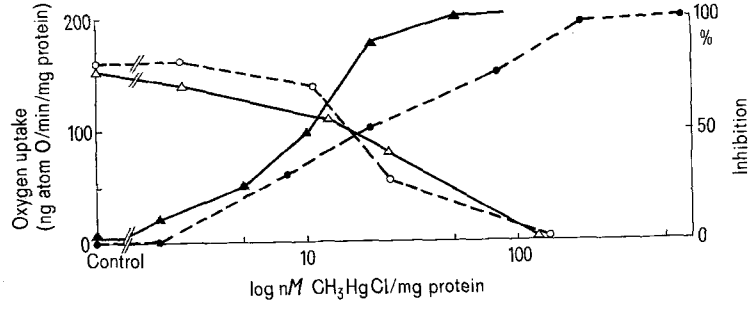

Methyl mercury effect on oxydase and deshydrogenase activities. ○—○, succinate oxydation; △—△, hydroxybutyrate oxydation; ●—●, succinate deshydrogenase activity (% inhibition); ▲—▲, D-3-hydroxybutyrate deshydrogenase activity (% inhibition).