the profile of protein kinase activity in experiments with high and low cAMP concentrations showed that association of protein kinase activity with cAMP receptors is possible with a low concentration of the ligand, and dissociation if its concentration is increased (Fig. 3). With a low cAMP concentration protein kinase activity was found in the peak of the dead volume with mol. wt. of 130 kilodaltons or more, whereas in the presence of a high cAMP concentration it was found in peaks with mol. wt. of about 110 and 65 kilodaltons. Catalytic subunits in these peaks are perhaps in the form of a complex with certain proteins that are not receptors for cAMP, which explains the difference in their molecular weight. The experimental results thus give grounds, in our opinion, for a number of suggestions concerning ontogenetic changes in cytoplasmic cAMP reception in the kidney. On the basis of estimation of the affinity constants and the results of gel filtration of protein kinases in the presence of a low cAMP concentration it can be concluded that reception of cAMP in each age group is effected mainly by regulatory subunits of one particular type that is characteristic of the given age. cAMP receptors are evidently mainly macromolecular complexes in which, besides regulatory subunits of cAMP-dependent protein kinases, there are also nonreceptor proteins. During maturation of kidney function the ability of the receptor complexes to dissociate under the influence of cAMP increases, and this may be connected with the development of sensitivity of the renal epithelium to the action of hormones.

LITERATURE CITED

1. G. Dlouga, I. Kršeček, and Yu. Natočin, Ontogeny of the Kidney [Russian translation], Leningrad (1981).
2. M. M. Pertseva, in: Abstracts of Proceedings of the 8th Conference on Evolutionary Physiology [in Russian], Leningrad (1982), p. 241.
3. P. Andrews, Biochem. J., 91, 222 (1964).
4. J. P. Charlton, C.-H. Huang, and L. C. Huang, Biochem. J., 209, 581 (1983).
5. S. O. Doskeland and D. Øgreid, Int. J. Biochem., 13, 1 (1981).
6. P. Greengard, Science, 199, 146 (1978).
7. F. J. Kuo and P. Greengard, J. Biol. Chem., 245, 4067 (1970).
8. P. C. Lee, D. Randolff, J. S. Schweppe, et al., J. Biol. Chem., 251, 914 (1976).
9. M. V. Nesterova, A. I. Glukhov, and E. S. Severin, Mol. Cell. Biochem., 49, 53 (1982).
10. H. E. Rosenthal, Anal. Biochem., 20, 525 (1967).
11. M. Yucel and I. Ozer, FEBS Lett., 125, 120 (1981).

CHANGES IN PURINE METABOLISM IN MOUSE MACROPHAGES DUE TO A NEW SYNTHETIC ANALOG OF MURAMYL DIPEPTIDE

V. Yu. Umanskii, A. M. Tarakhovskii, and T. M. Andronova

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Recently the attention of many investigators has been concentrated on the study of the immunomodulating properties of muramyl dipeptide (N-acetyl-muramyl-L-alanyl-D-isoglutamine; MDP), a minimal chemical component of the glycopeptide of bacterial cell walls, which exhibits adjuvant activity [4]. This preparation increases the cytolytic activity of mouse peritoneal macrophages in vivo and in vitro against both bacteria [14] and tumor cells [7]. It was shown previously that N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP), a new analog of MDP, has a marked antitumor action, and with respect to several parameters,

R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev. M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.)

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TABLE 1. Effect of GMDP on AD and 5-N Activity in Mouse Macrophages (M ± m)

Enzyme	Control	GMDP
AD	117,8±11,8 (9)	94,6±7,6 (11)
5-N	39,1±0,9 (18)	8,4±0,4* (26)

Legend. Here and in Table 2, *P < 0.05 compared with control; number of experiments given in parentheses.

its biological activity is much greater than that of MDP [2]. Meanwhile metabolic changes due to the action of MDP and GMDP have not been adequately studied. It has recently been shown that an important role in macrophage activation is played by changes in activity of enzymes of purine metabolism, namely adenosine deaminase (AD) and 5-nucleotidase (5-N), which regulate the intracellular adenosine concentration [13].

The aim of this investigation was to study the action of GMDP and its derivatives in vitro on AD and 5-N activity in mouse peritoneal macrophages.

EXPERIMENTAL METHOD

BALB/c mice aged 1.5-2 months were used. GMDP and its structural analogs were synthesized by methods described previously [3]. Peritoneal macrophages were obtained by the standard method from mice into which 3 ml of nutrient broth was injected intraperitoneally 72 h before the beginning of the experiment. The macrophages thus obtained were cultured in plastic Petri dishes (diameter 40 mm) under the conditions specified in [8]. GMDP and its analogs were added to the medium in concentrations of 0.01 to 10 μg/ml. After the end of incubation the monolayer of macrophages, washed to remove medium, was treated with 1 ml of lytic solution containing 20 mM Tris-HCl, 5 mM $MgSO_4$, pH 7.4. AD and 5-N activity in the resulting lysate of macrophages was determined with the aid of [^{14}C]adenosine, [^{14}C]-AMP, and ascending paper chromatography in a micromodification of the method in [1]. AD activity was expressed in nanomoles inosine and hypoxanthine/min/mg protein; 5-N activity was expressed in nanomoles adnosine, inosine, and hypoxanthine/min/mg protein. Protein was determined by Lowry's method [10].

EXPERIMENTAL RESULTS

After incubation of the macrophages for 24 h in the presence of GMDP (1 μg/ml) a tendency was observed for AD activity to fall, but this was not significant (Table 2). Meanwhile 5-N activity decreased significantly (by 4.7 times) compared with the control under these conditions.

An important role in the action of GMDP on 5-N activity in macrophages is played by the duration of their incubation together (Fig. 2). As early as 2 h after the beginning of incubation, activity of the enzyme began to fall, to reach a minimum by 24 h. Treatment of the macrophages with GMDP in an increasing concentration led to a gradual decrease in 5-N activity (Fig. 1). Changes under the influence of GMDP were observed in a concentration of only 0.01 μg/ml, and the maximal effect was obtained with GMDP in concentrations of 1 and 5 μg/ml. The action of GMDP is determined by the structure of the substance tested (Table 2). Neither D-D- nor L-L-isomers of GMDP (1 μg/ml) were found to affect 5-N activity in macrophages. Meanwhile the analog with a double dipeptide region $GM(DP)_2$ inhibited activity of the enzyme by 1.6 times, although by a lesser degree than GMDP. The presence of alanyl-D-isoglutamine in the structure of the GMDP dipeptide is essential for the activating action of the preparation to be exhibited, since N-acetylglucosaminyl-N-acetylmuramine did not affect the enzymic activity of the test cells.

The results are evidence that GMDP causes a significant decrease in 5-N activity in mouse peritoneal macrophages in vitro. The decrease in activity of this enzyme in the macrophages under the influence of various bacterial agents has been demonstrated by other workers also [6, 9, 13]. The change in 5-N activity thus observed has also been regarded by many workers as an indicator of activation of the macrophages, irrespective of whether it was due to the introduction of an infectious agent or to the appearance of tumor cells [11]. 5-N is an exoenzyme and hydrolyzes extracellular AMP and facilitates intracellular transport of the adenosine thus formed [5]. During incubation of macrophages with GMDP the conditions are therefore created for a decrease

TABLE 2. Effect of GMDP and Its Structural Analogs on 5-N Activity in Mouse Macrophages (M ± m)

Preparation	Activity of 5-N
Control	39,1±0,9 (18)
GMDP	8,4±0,4* (26)
N-acetylglucosaminyl-N-acetylmuramyl-D-alanyl-D-isoglutamine (D-D-GMDP)	36,6±1,8 (5)
N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-L-isoglutamine (L-L-GMDP)	38,7±1,7 (5)
N-acetylglucosaminyl-N-acetylmuramine (GM)	37,5±1,6 (5)
N-acetylglucosaminyl-N-acetylmuramyl-[alanyl-D-isoglutamine]$_2$ [GM(DP)$_2$]	24,6±2,2* (5)

Legend. Concentration of compounds 1 μg · ml^{-1}.

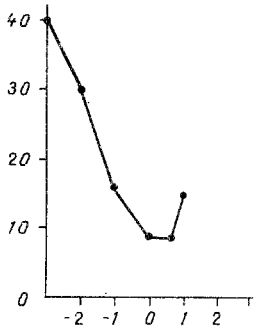

Fig. 1

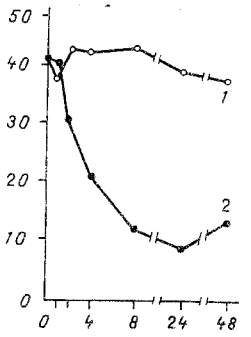

Fig. 2

Fig. 1. Action of GMDP in various concentrations on 5-N activity in mouse macrophages. Duration of incubation 24 h. Abscissa, log of GMDP concentration (in μg/ml); ordinate, enzyme activity (in nanomoles/min/mg protein).

Fig. 2. 5-N activity in mouse macrophages as a function of duration of their incubation with GMDP (1 μg/ml). Abscissa, duration of incubation (in h); ordinate, enzyme activity (in nanomoles/min/mg protein). 1) Control, 2) GMDP.

in the intracellular concentration of adenosine, an inhibitor of function of immunocompetent cells, including macrophages [12].

An important role in the mechanism of activation of macrophages under the influence of GMDP may therefore be played by lowering of the 5-N activity in these cells.

LITERATURE CITED

1. N. P. Dmitrenko, S. V. Komissarenko, and V. Yu. Umanskii, Dokl. Akad. Nauk SSSR, 251, 251 (1981).
2. V. T. Ivanov, Vopr. Med. Khimii, No. 3, 23 (1984).
3. L. I. Rostovtseva, T. M. Andronova, V. P. Mal'kova, et al., Bioorg. Khim., 7, 1843 (1981).

4. A. Adam, J.-F. Petit, N. Lefrancier, et al., Molec. Cell. Biochem., 41, 27 (1981).
5. J. R. S. Arch and E. A. Newsholme, Essays Biochem., 14, 82 (1978).
6. I. Bursuker and R. Goldman, J. Reticuloend. Soc., 26, 205 (1979).
7. L. Chedid, M. Parant, F. Parant, et al., Proc. Natl. Acad. Sci. USA, 74, 2089 (1977).
8. R. B. Johnston, C. A. Godzik, and Z. A. Cohn, J. Exp. Med., 148, 115 (1978).
9. M. L. Karnovsky and J. K. Kadzins, J. Immunol., 121, 809 (1978).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. P. S. Morahan, P. J. Edelson, and K. Gass, J. Immunol., 125, 1312 (1980).
12. A. Ohtani, Y. Kumazawa, H. Fujisawa, et al., J. Reticuloend. Soc., 32, 189 (1982).
13. C. E. Pietrangeli, E. Skamene, P. J. Edelson, et al., Infect. Immun., 32, 1206 (1981).
14. M. Uemiya, K. Sugimura, T. Kusama, et al., Infect. Immun., 24, 83 (1979).

HORMONAL REGULATION OF ANABOLIC METABOLISM AND EFFICIENCY OF PROTEIN UTILIZATION IN THE EARLY POSTNATAL PERIOD

L. F. Adigamov, L. I. Smirnova,
V. D. Nifontov, and M. P. Chernikov

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There is only limited information in the literature on the study of production of various anabolic hormones in early ontogeny and the efficiency of protein assimilation [4, 7, 8]. However, such research could fill in the gaps in our knowledge of the mechanisms of regulation of protein metabolism in the early stages of development of the organism, when the efficiency of protein utilization in man and various species of mammals is considerably higher than in the adult [4, 8].

In connection with the above facts it was decided to study on account of which endogenous anabolic regulatory systems this high level of protein utilization is maintained. The aim of the present investigation was to study hormonal regulators of protein metabolism, namely somatotrophin (STH), 11-hydroxycorticosteroids (11-HCS), thyroxine, and triiodothyronine, and the efficiency of protein utilization at different stages of the neonatal period.

EXPERIMENTAL METHOD

11-HCS, thyroxine, and triiodothyronine were determined in blood serum, STH in the pituitary glands of male rats at the following stages of the early postnatal period: the transition from embryonic to milk feeding (5th day of postnatal life), milk feeding (15th day), transition to ordinary feeding (30th day).

To determine STH, the freshly isolated pituitary gland was homogenized in distilled water on a Teflon homogenizer at 600 rpm for 30 sec. The homogenate was transferred to a tube and covered with 7.5% polyacrylamide gel (pH 8.6). Electrophoresis was carried out in the ÉF-1 apparatus (USSR) in 0.05 M Tris-glycine buffer. After the completion of electrophoresis the column of gel was removed from the tube and stained with 0.1% Coomassie blue in 10% TCA for 30 min. After staining, the gel was washed to remove excess of dye with a mixture of acetic acid–98% ethanol–water (75:200:725) and subjected to densitometry on the ERI-65 instrument.

Laboratory of Protein Metabolism, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 687-689, June, 1985. Original article submitted April 30, 1984.

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