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INTERACTION OF ATP WITH A MACROMOLECULAR TRANSLOCATION INHIBITOR OF THE NUCLEAR BINDING OF "ACTIVATED" RECEPTOR-GLUCOCORTICOID COMPLEX

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SUMMARY

Incubation of 1-5 mM ATP with nuclei and partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex from rat liver cytosol had no significant effect on association of the activated complex with the nuclei. However, when the nuclear uptake was reduced by the macromolecular translocation inhibitor in the rat liver cytosol, addition of 5 mM ATP restored the uptake to the level without inhibitor. ADP and AMP as well as other nucleotides tested could not overcome the inhibitory effect of macromolecular inhibitor.

The action of steroid hormones on their target cells seems to be regulated in a complicated manner at many regulatory points (1-10). One of these points is suggested to be a step in translocation or binding of the already "activated" receptor-steroid complex to the nucleus, chromatin or DNA (10). Several factors have been found to influence this step (10-20); namely, macromolecular translocation inhibitor (11-16), pyridoxal 5'-phosphate (17) and a low-molecular weight translocation modulator(s) in rat liver cytosol (18-20). The low-molecular-weight translocation modulator(s) inhibited nuclear uptake of "activated" receptor-glucocorticoid complex, but it decreased the inhibitory effect of the macromolecular translocation inhibitor when the two were added together (18-20). The low-molecular-weight translocation modulator(s) consists of several components judging from its elution pattern of a Sepadex G-10 column (19). The ranges of molecular weights of the components (19) suggested the possible participation of various nucleotides in this step. We report in this

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paper that ATP has a specific effect in decreasing the inhibitory effect of macromolecular translocation inhibitor.

MATERIALS AND METHODS

Materials. [^{3}H]Triamcinolone acetonide (25 Ci/mmol) were obtained from the Radiochemical Centre. Nucleotides were obtained from Sigma Chemical Co. All other chemicals (reagent grade) were purchased from Wako Pure Chemical Industries, Ltd.

Preparation of macromolecular translocation inhibitor. Male albino Donryu rats (55g-65g) obtained from Kitayama Rabes Corp. (Kyoto, Japan) were kept under our standard laboratory conditions (21-24). The rats, weighing 180g to 200g, were adrenalectomized bilaterally 3 days before sacrifice and were given 0.9% NaCl solution to drink after the operation (21-24). Animals were killed by decapitation and their livers were perfused with 30 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.55) containing 250 mM sucrose, 25 mM KCl, 3 mM $MgCl_2$, and 1 mM 2-mercaptoethanol (homogenization buffer)(21). The liver was then homogenized in 2 volumes of the buffer. The cytoplasmic supernatant fraction (cytosol) was prepared from the homogenate by centrifugation at 105,000 x g for 60 min at 2°C. The liver cytosol was incubated at 37°C for 30 min, then cooled in an ice-bath for 10 min and centrifuged at 8,500 x g for 20 min (20). The resulting supernatant with no remaining specific binding capacity for [^{3}H]triamcinolone acetonide was dialyzed against homogenization buffer, and used as macromolecular translocation inhibitor (20).

Partial purification of the "activated" receptor-glucocorticoid complex. The "activated" receptor-[^{3}H]triamcinolone acetonide complex was partially purified by a modification (20) of the method of Climent et al. (25).

Preparation of nuclei. Nuclei were obtained from the liver of adrenalectomized rats as reported previously (20) and were resuspended at a final concentration of 2 x 10^7/ml in homogenization buffer with 1 mM Na_2EDTA (20).

Nuclear binding assay. The assay mixture consisted of partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex (10 µl, 50,000 cpm), 2 x 10^6 nuclei (100 µl) and various concentrations of nucleotides dissolved in the homogenization buffer with or without a constant amount (4 mg protein) of macromolecular translocation inhibitor. The volume was made up to 500 µl with homogenization buffer (20). The mixture in an Eppendorf microtube (1.5 ml)(Eppendorf Gerätebau Netheler + Hinz GmbH, Hamburg, West Germany), was incubated at 0°C for 90 min, then centrifuged at 8,000 x g for 2 min at 4°C (20). The precipitate was washed 3 times with 1.4 ml of homogenization buffer. The resulting nuclear pellet was solubilized in 0.5 ml of NCS tissue solubilizer. Radioactivity of the nuclear pellet was measured as described elsewhere (21).

Protein determination. Protein was determined by a modification (26) of the method of Lowry et al. (27) with bovine serum albumin as a standard.

RESULTS

Use of homogeneous populations of "activated" receptor-[^{3}H]triamcinolone acetonide complexes (20,25,28) which show no reversal to the non-activated

state, obtained by phosphocellulose column chromatography (20,25,28), has simplified studies on the interation of "activated" complexes with nuclear acceptors (20,28). The effect of ATP at 0°C on nuclear uptake of the "activated" complex in shown is Fig.1-A. ATP concentrations up to 5 mM did not have any appreciable effect on nuclear uptake in the absence of the macromolecular translocation inhibitor. Addition of the macromolecular translocation inhibitor (4 mg protein) to the assay mixture decreased nuclear uptake to about 50% of the control value. When assay mixtures with macromolecular translocation inhibitor were incubated with different concentrations (1-5 mM) of ATP at 0°C for 90 min, their decreased nuclear uptake was restored to nearly the control level. Unlike ATP, ADP and AMP at concentrations of 1-5 mM had no effect (Fig. 1-B, 1-C). These results suggest that the effect of 1-5 mM ATP in enhancing nuclear uptake of "activated" receptor-steroid complex is due to prevention of the inhibitory action of the macromolecular translocation inhibitor.

Next we examined the effect of cAMP in the same way (Fig. 1-D). cAMP at concentrations of 10^{-8}M to 10^{-6}M had little effect on nuclear uptake with or without the macromolecular inhibitor. When the macromolecular inhibitor and cAMP at a concentration of 10^{-5}M (about 100 times the physiological concentration) were mixed, however, enhanced nuclear uptake was observed (Fig. 1-D). The effects of other nucleotides, such as UTP, ITP, TTP, CTP and GTP, were also investigated (Table 1). Unlike ATP, these nucleotides at concentrations of 3 mM were not effective. However, 5 mM CTP (about 100 times the physiological concentration) increased nuclear uptake in the same manner as cAMP, and 10 mM ATP increased nuclear uptake to above the control value, irrespective of the presence of macromolecular inhibitor (data not shown). The reason for these effects is unknown. The effect of ATP at physiological concentrations seems to be fairly specific, when compared with the effects of other nucleotides.

DISCUSSION

ATP has been suggested to be involved in regulation of steroid receptor

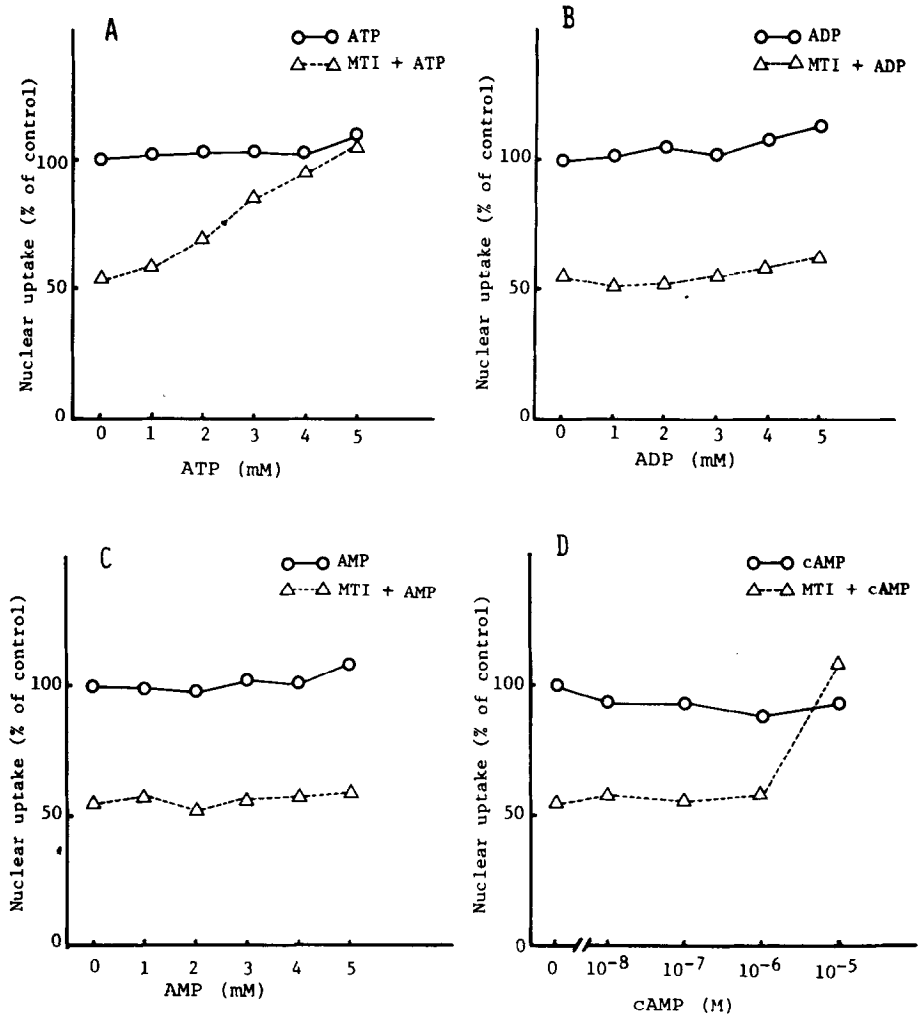

Fig. 1. Effects of various concentrations of nucleotides on nuclear uptake of partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex. Increasing amounts of ATP (A), ADP (B), AMP (C) and cAMP (D) were added to the nuclear uptake assay mixture with (o) or without (Δ), the macromolecular translocation inhibitor (MTI)(4 mg protein). The mixture was made up to 0.5 ml with homogenization buffer and incubated at 0°C for 90 min. Other procedures were as described in the Materials and Methods. Values are expressed as percentages of the value in the control containing buffer, instead of nucleotide and macromolecular inhibitor. Values are means of duplicate determinations in four separate experiments.

activity. Munck and his coworkers (29,30) reported that the extent of specific corticosteroid binding by thymus cells was linearly proportional to the cellular ATP level. The level of nuclear binding of corticosteroids is also known to be related with the cellular ATP level (31). Furthermore nuclear binding of

TABLE I.

INTERACTION OF MACROMOLECULAR TRANSLOCATION INHIBITOR WITH NUCLEOTIDES

Nucleotide	Nuclear uptake (% of control)
None	53.6
UTP	53.0
ITP	49.4
TTP	52.5
CTP	58.3
GTP	52.0

UTP, ITP, TTP, CTP and GTP were added at a concentration of 3 mM with the macromolecular translocation inhibitor. Nuclear uptake of partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex was measured. Nuclear binding was determined as described in Fig. 1 and Materials and Methods. Values are means of dupulicate determinations in two separate experiments.

the receptor-estrogen complex was enhanced by incubating the nuclei with ATP (32). Pratt and coworkers (1,2) suggested that glucocorticoid receptors can be activated to a glucocorticoid binding state by an ATP-dependent phosphorylation mechanism in L cells (2) and thymus cells (1). The "activated" avian receptor-progesterone complex was found to bind to ATP-Sepharose (33,34) and ATP at 4°C was found to reduce transformation of a non-activated receptor-glucocorticoid complex to an activated form (35). However, the presence of ATP and an ATP regenerating system did not change the nuclear uptake of receptor-glucocorticoid complex in rat liver cytosol (10,36).

In our nuclear uptake system (20), it is possible to study the translocation mechanism of receptor-glucocorticoid complex to nuclei in a simplified manner using homogeneous populations of activated complexes showing no reversal to the non-activated state and negligible contamination with components that affect nuclear uptake. This paper reports that ATP has no effect on nuclear uptake of the "activated" receptor-glucocorticoid complex, but overcomes the

inhibitory action of the macromolecular translocation inhibitor. This effect was fairly specific for ATP at physiological concentrations. The mechanism of the effect of ATP is unknown. Because of the high affinity of the activated receptor complex for ATP, it seems probable that ATP-bound activated receptor-glucocorticoid complex would be resistant for the inhibitory action of the macromolecular inhibitor, although it is possible that ATP interacts directly with the macromolecular inhibitor. Further investigations of the effects and mechanisms of action of various nucleotides and cyclic nucleotides on the nuclear translocation step are in progress.

REFERENCES

1. Sando, J. J., Nielsen, C. J. and Pratt, W. B. (1977) J. Biol. Chem. 252, 7579-7582.
2. Sando, J. J., La Forest, A. C. and Pratt, W. B. (1979) J. Biol. Chem. 254, 4772-4778.
3. Kanazir, D., Ribarac-Stepić, N., Trajković, D., Blečić, G. and Radojčić, M. (1979) J. Steroid Biochem. 11, 389-400.
4. Atger, M. and Milgrom, E. (1976) J. Biol. Chem. 251, 4758-4762.
5. Bailly, A., Sallas, N. and Milgrom, E. (1977) J. Biol. Chem. 252, 858-863.
6. Cake, M. H., Goidl, J. A., Parchman, L. G. and Litwack, G. (1976) Biochem. Biophys. Res. Commun. 71, 45-52.
7. Cake, M. H. and Litwack, G. (1978) Eur. J. Biochem. 82, 97-103.
8. Cake, M. H. and Litwack, G. (1975) Biochem. Biophys. Res. Commun. 66, 828-835.
9. Higgins, S. J., Rousseau, G. G., Baxter, J. D. and Tomkins, G. M. (1973) J. Biol. Chem. 248, 5866-5872.
10. Kalimi, M., Beato, M. and Feigelson, P. (1973) Biochemistry 12, 3365-3371.
11. Atger, M. and Milgrom, E. (1978) Biochim. Biophys. Acta 539, 41-53.
12. Chamness, G. C., Jennings, A. W. and McGuire, W. L. (1974) Biochemistry 13, 327-331.
13. Liu, S. H. and Webb, T. E. (1977) Cancer Res. 37, 1763-1767.
14. Milgrom, E. and Atger, M. (1975) J. Steroid Biochem. 6, 487-492.
15. Simons, S. S., Jr. (1977) Biochim. Biophys. Acta 496, 339-348.
16. Simons, S. S., Jr., Martinez, H. M., Garcea, R. L., Baxter, J. D. and Tomkins, G. M. (1976) J. Biol. Chem. 251, 334-343.
17. Cake, M. H., DiSorbo, D. M. and Litwack, G. (1978) J. Biol. Chem. 253, 4886-4891.
18. Isohashi, F., Terada, M., Tsukanaka, K., Nakanishi, Y., Fukushima, H. and Sakamoto, Y. (1979) Proc. Jpn. Cancer Assoc., The 38th Annual Meeting, Abst. 231
19. Isohashi, F., Terada, M. and Sakamoto, Y. (1980) Seikagaku, 52, 1009-1010.
20. Isohashi, F., Terada, M., Tsukanaka, K., Nakanishi, Y. and Sakamoto, Y. (1980) J. Biochem. 88, 775-781.
21. Isohashi, F., Terada, M., Nakanishi, Y. and Sakamoto, Y. (1976) Cancer Res. 36, 4382-4386.
22. Isohashi, F., Tsukanaka, K., Terada, M., Nakanishi, Y., Tani, S. and Sakamoto, Y. (1978) Cancer Res. 38, 4243-4245.
23. Isohashi, F., Tsukanaka, K., Terada, M., Nakanishi, Y., Fukushima, H. and Sakamoto, Y. (1979) Cancer Res. 39, 5132-5135.

24. Isohashi, F., Tsukanaka, K., Terada, M., Nakanishi, Y., Tani, S. and Sakamoto, Y. (1980) Cancer Res. 40, 877-881.
25. Climent, F., Bugany, H. and Beato, M. (1976) FEBS Lett. 66, 317-321.
26. Hartree, E. F. (1972) Anal. Biochem. 48, 422-427.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
28. Atger, M. and Milgrom, E. (1976) Biochemistry, 15, 4298-4304.
29. Munck, A. and Brinck-Johnsen, T. (1968) J. Biol. Chem. 243, 5556-5565.
30. Bell, P. A. and Munck, A. (1973) Biochem. J. 136, 97-107.
31. Sloman, J. C. and Bell, P. A. (1976) Biochim. Biophys. Acta 428, 403-413.
32. King, R. J. B., Gordon, J., Mark, J. and Steggles, A. W. (1971) in Basic Actions of Sex Steroids on Target Organs (Karger, Basel), 21-43.
33. Moudgil, V. K. and Toft, D. O. (1975) Proc. Nat. Acad. Sci. USA 72, 901-905.
34. Miller, J. B. and Toft, D. O. (1978) Biochemistry 17, 173-177.
35. John, J. K. and Moudgil, V. K. (1979) Biochem. Biophys. Res. Commun. 90, 1242-1248.
36. Beato, M., Kalimi, M., Beato, W. and Feigelson, P. (1974) Endocrinology 94, 377-387.