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REGULATION OF NUCLEAR UPTAKE OF "ACTIVATED" RECEPTOR-GLUCOCORTICOID COMPLEX BY PYROPHOSPHATE

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Nuclear uptake of partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex from rat liver cytosol in vitro was significantly increased by incubation with pyrophosphate, reaching a maximum with 2-7 mM pyrophosphate, but with higher concentrations of 9-10 mM pyrophosphate it decreased gradually to below the control level. When nuclear uptake of "activated" complex was inhibited by macromolecular translocation inhibitor, ATP could overcome the inhibitory effect of macromolecular inhibitor, while 1-5 mM pyrophosphate could not enhanced nuclear uptake. Addition of 1-5 mM phosphate did not affect nuclear uptake or the action of the macromolecular translocation inhibitor.

Binding or translocation of already "activated" receptor-steroid complex to the nucleus, chromatin or DNA is reported to be inhibited by macromolecular translocation inhibitor (1-6), pyridoxal 5'-phosphate (7), and some other chemicals such as rifamycin AF/013 (8,9), o-phenanthroline (8,9) and aurintricarboxylic acid (10). In addition to these inhibitors, we reported that low-molecular-weight translocation modulators from rat liver cytosol regulate nuclear uptake of "activated" receptor-glucocorticoid complex in a complicated manner (11,12). ATP, which is one of the components of low-molecular-weight translocation modulators, has a specific effect in decreasing the inhibitory effect of macromolecular translocation inhibitor, but does not influence the nuclear uptake in the absence of macromolecular inhibitor (12). We report in this paper that pyrophosphate at 2-7 mM significantly enhanced nuclear uptake of "activated" receptor-glucocorticoid complex, but was not apparently effective in the presence of macromolecular translocation inhibitor. A preliminary account of part of this work has been reported (13).

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MATERIALS AND METHODS

Animals. Male albino Donryu strain rats (180-200 g) were adrenalectomized bilaterally 3 days before sacrifice. They were fed laboratory chow, given 0.9% NaCl solution to drink and kept under our standard labolatory condition after surgery (14-17).

Materials. [^{3}H]Triamcinolone acetonide (26 Ci/mmol) was purchased from the Radiochemical Center. ATP and bovine serum albumin were obtained from Sigma Chemical Co. All other chemicals (reagent grade) were purchased from Wako Pure Industries, Ltd.

Preparation of partially purified "activated" receptor-glucocorticoid complex. The "activated" receptor-[^{3}H]triamcinolone acetonide complex was partially purified by a modification of the method of Climent et al. (18) as described in details elsewhere (11).

Preparation of macromolecular translocation inhibitor. The macromolecular translocation inhibitor was prepared from the livers of adrenalectomized rats as described previously (11,12). The absence of remaining specific binding capacity for [^{3}H]triamcinolone acetonide was confirmed by the method described elsewhere (14).

Effects of pyrophosphate, phosphate and ATP on nuclear uptake (or chromatin binding) of partially purified "activated" receptor-glucocorticoid complex. Nuclei were prepared from the livers of adrenalectomized rats by the method of Beato et al. (19). Nuclear uptake of "activated" receptor-glucocorticoid complex was determined as follows. The assay mixture (pH 7.4 at 4°C) consisted of 50 mM Tris-HCl, 250 mM sucrose, 4 mM $MgCl_2$, 25 mM KCl, 0.2 mM Na_2EDTA, 1 mM 2-mercaptoethanol, 2 x 10^6 nuclei and partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex (50,000 cpm), and when specified, various concentrations of pyrophosphate, phosphate or ATP and macromolecular translocation inhibitor (4 mg protein) were added to the assay mixture. The final volume of the assay mixture was 500 µl. The mixture was incubated for 90 min at 0°C and centrifuged at 8,500 x g for 2 min. The precipitate was washed 3 times with 1.4 ml buffer (pH 7.4 at 4°C) containing 50 mM Tris-HCl, 250 mM sucrose, 4 mM $MgCl_2$, 25 mM KCl, 1 mM Na_2EDTA and 1 mM 2-mercaptoethanol, and then its radioactivity was counted as described previously (11,12,14).

Chromatin was prepared from the livers of adrenalectomized rats by the method of Spersberg and Hnilica (20) and suspended in buffer (pH 7.4 at 4°C) containing 2 mM Tris-HCl and 0.1 mM Na_2EDTA. Chromatin suspension (20 µg DNA) was added to the assay mixture (pH 7.4 at 4°C) containing 10 mM Tris-HCl, 10% glycerol (v/v), 4 mM $MgCl_2$, 25 mM KCl, 0.5 mM Na_2EDTA, 1 mM 2-mercaptoethanol, 4 mg bovine serum albumin and partially purified "activated" receptor-[^{3}H]-triamcinolone acetonide complex (50,000 cpm). The final volume of the assay mixture was 500 µl. After incubation for 90 min at 0°C, the assay mixture was centrifuged for 10 min and then washed 3 times with 1.4 ml buffer (pH 7.4 at 4°C) containing 10 mM Tris-HCl, 10% glycerol (v/v), 4 mM $MgCl_2$, 25 mM KCl, 0.5 mM Na_2EDTA, 1 mM 2-mercaptoethanol and 8 mg/ml bovine serum albumin. Other procedures were as for nuclear uptake assay.

DNA and protein determination. DNA was determined with diphenylamine by the method of Burton (21). Protein was determined by a modification (22) of the method of Lowry et al. (23) with bovine serum albumin as a standard.

RESULTS

"Activated" receptor-[^{3}H]triamcinolone acetonide complex purified about 3,000-5,000-fold by phosphocellulose column chromatography (11), which did not contain any appreciable amount of translocation modulators, was used throughout

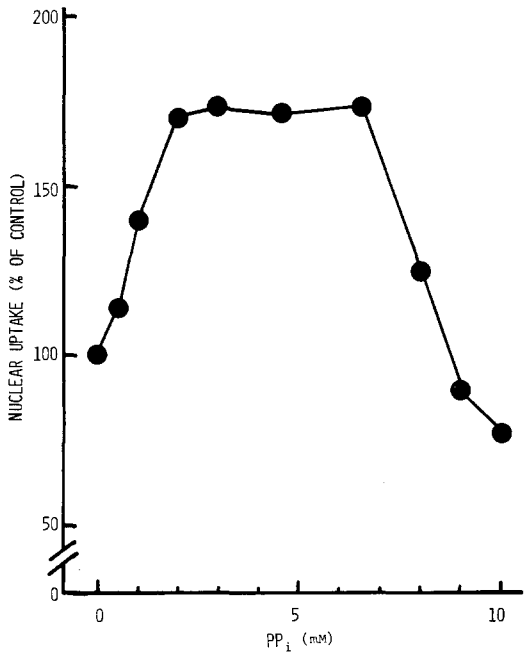

Fig. 1. Effects of various concentrations of pyrophosphate on nuclear uptake of partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex. Increasing amounts of pyrophosphate were added to the assay mixture. Nuclear uptake was assayed as described in the MATERIALS AND METHODS. Values are percentages of the value in the control not containing pyrophosphate. Values are means of duplicate determinations in three separate experiments.

the experiments. The effect of pyrophosphate on nuclear uptake of "activated" receptor-glucocorticoid complex at 0°C is shown in Fig. 1. Progressive addition of pyrophosphate steadily enhanced nuclear uptake of the "activated" complex to a maximum (about 1.7-fold) with 2 mM pyrophosphate and then uptake remained at a plateau up to 7 mM pyrophosphate. Further addition of pyrophosphate gradually decreased nuclear uptake to below the control level with 9-10 mM pyrophosphate. Similar results were obtained with chromatin as acceptor instead of nuclei (data not shown). When nuclei or chromatin were preincubated at 0°C for 30 min with various concentrations of pyrophosphate, washed twice and then tested for ability to bind to the "activated" receptor-glucocorticoid complex, it was found that preincubation with various concentrations of pyrophosphate had no detectable effect on the binding (data not shown), suggesting that pyrophosphate did not act through acceptor sites, such as nuclei or chromatin, or that pyrophosphate interacted very weakly with acceptors. As we reported previously (12), addition of the macromolecular translocation inhibitor

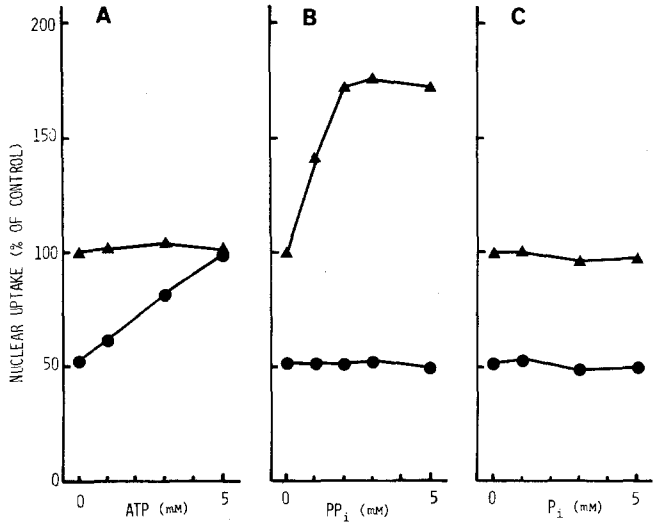

Fig. 2. Effects of ATP (A), pyrophosphate (B) and phosphate (C) on nuclear uptake of partially purified "activated" receptor-[^{3}H]triamcinolone acetonide complex. Various concentrations of ATP, pyrophosphate and phosphate were added to the assay mixture with (●) or without (▲) the macromolecular translocation inhibitor (4 mg protein). Other procedures were as described in the MATERIALS AND METHODS. Values are percentages of the value in the control not containing ATP, pyrophosphate, phosphate and the macromolecular translocation inhibitor. Values are means of duplicate determinations in three separate experiments.

(4 mg protein) to the assay mixture decreased nuclear uptake of "activated" receptor-glucocorticoid complex to about 50% of the control value, and the decreased nuclear uptake could be restored to nearly the control level by addition of 5 mM ATP (12). Similar results (Fig. 2-A) were obtained in the conditions of the assay mixture (pH 7.4 at 4°C, 4 mM $MgCl_2$, see "MATERIALS AND METHODS") instead of the conditions used previously (12) and with chromatin instead of nuclei. In contrast to ATP, pyrophosphate at concentrations of 1-5 mM had no effect on the inhibitory action of the macromolecular inhibitor (Fig. 2-B). Addition of phosphate to the assay mixture had no detectable effect on nuclear uptake either in the absence or presence of the macromolecular inhibitor (Fig. 2-C).

DISCUSSION

The present experiments show that pyrophosphate at concentrations of up to 7 mM increases nuclear uptake of already "activated" receptor-glucocorticoid complex, but that higher concentrations of 9-10 mM decrease nuclear uptake to below the control level. In the presence of macromolecular translocation inhibitor, 1-5 mM pyrophosphate did not enhance nuclear uptake, in contrast to ATP.

The exact mechanism of interaction of pyrophosphate and ATP with already "activated" receptor-glucocorticoid complex, nuclei, chromatin or macromolecular translocation inhibitor is unknown.

The effect of pyrophosphate and ATP reported in this paper is interesting, considering that avian progesterone receptor may have enzymatic activity for the ATP-PPi exchange reaction (24) and that the "activated" receptor-steroid complex has high affinity for ATP (25-27). Whether glucocorticoid receptor contains enzymatic activity or not, the present results are suggestive that pyrophosphate and ATP might be involved in some aspects of receptor function. Further investigations on the effect of pyrophosphate and ATP on the nuclear translocation step, including the enzymatic activity of the "activated" receptor-[^{3}H]triamcinolone acetonide complex, are in progress.

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