Enzyme induction in rat liver: The effects of Be\(^{2+}\) *in vivo*

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Rats given an LD\(_{50}\) dose of Be\(^{2+}\) showed reduced activities of ornithine decarboxylase and tyrosine aminotransferase in liver in response to dexamethasone induction. Control fed animals showed 'superinduction'. Be\(^{2+}\) also inhibited the uptake of \([^{3}H]\)orotic acid into rapidly labelled RNA of ribonucleoprotein particles extracted from liver nuclei in isomolar solutions at pH 8.0. Consistent with inhibition of cytoplasmic protein kinase reported previously (Kaser et al., 1980), the uptake of \([^{32}P]\)Pi into proteins in the ribonucleoprotein particles was also diminished.

A biochemical basis for beryllium toxicity has been sought since the 1950s (see Reiner, 1970). The metal is administered intravenously as a 1:1 molar mixture of BeSO\(_4\):sulphosalicylic acid which is ultimately concentrated in the liver. The most obvious consequence is an inhibition of enzyme induction in resting and regenerating liver (Witschi & Marchland, 1971; Witschi, 1970). A number of enzymes are inhibited by 1-10 \(\mu\)M Be\(^{2+}\) *in vitro*: these are now known to be phosphorylated in their catalytic sites (see Reiner, 1970). Be\(^{2+}\) administration in the LD\(_{50}\) range has been found to inhibit liver cytoplasmic protein kinase(s) by about 40% and to diminish histone phosphorylation in resting and regenerating liver similarly (Kaser et al., 1980). The relationship between the inhibition of enzyme induction and of nuclear protein phosphorylation has now been examined, using ornithine decarboxylase (EC 4.1.1.17) and tyrosine aminotransferase (EC 2.6.1.5) as models, since glucocorticoid induction does not involve detectable stimulation of cyclic AMP-dependent protein kinases(s). Transcription was followed in ribonucleoprotein particles released from liver nuclei by isomolar solutions at pH 8.0 (Louis & Sekeris, 1976). Induction was found to be inhibited by Be\(^{2+}\) *in vivo*, as was the uptake of \([^{3}H]\)orotic acid by the ribonucleoprotein particles from dexamethasone-treated animals. Increased enzyme activity in control fed rats in the presence of Be\(^{2+}\) suggested that induction of the protease(s) catabolizing ornithine decarboxylase and tyrosine aminotransferase was also diminished by the metal.

Methods

200- to 400-g male Wistar rats were used. Food and water were available ad lib; the animal house was lit between 0600 and 2000 h.
The operative procedures have been reported previously (Thrower & Ord, 1974).

**Enzyme assays.** Livers were homogenized and ornithine decarboxylase and tyrosine aminotransferase measured in the 100 000-g supernatant as described elsewhere (Thrower & Ord, 1974, except that the final ornithine concentration was raised to 2 mM; Granner & Tomkins, 1970).

**Ribonucleoprotein particles.** Nuclei were prepared in 2.2 M sucrose/10 mM MgCl₂/50 mM Tris-HCl, pH 7.4, and ribonucleoprotein particles were isolated in isomolar solutions at pH 8.0 (Louis & Sekeris, 1976). The preparations were sedimented through sucrose gradients and the radioactivities of the RNA and protein determined. [³²P]Pi (100 μCi/100 g body wt., intramuscularly) was given to the rats for 21 h and [³H]orotic acid (50 μCi/100 g, intramuscularly) for 15 min before death.

Isotopes were from the Radiochemical Centre, Amersham, Bucks., HP7 9LL, U.K.

**Results and Discussion**

**Enzymic induction**

Be²⁺ administration reduced the induction of ornithine decarboxylase in rat livers by dexamethasone (Table 1A); in confirmation of earlier observations (Witschi & Marchand, 1971), the effect was more evident if the metal was given to the rats overnight, rather than coincident with the inducing agent. In contrast, in control rats which did not receive the steroid, and which ate during the night, the levels of activity of both ornithine decarboxylase (Table 1A) and tyrosine aminotransferase (Table 1C) were higher in animals which had been given Be²⁺ the night before than in normal animals. This enhancement by Be²⁺ was shown even in adrenalectomized rats (Table 1D). 0.1 mM Be²⁺ did not affect the activity of either enzyme *in vitro*.

Tyrosine aminotransferase levels were elevated by dexamethasone administration, and although the activity was further increased in animals which had received Be²⁺, inducing effects produced by the steroid were markedly diminished in the Be²⁺-treated rats (Table 1C; ratio of activities in induced:non-induced without Be²⁺, 3.84; with, 1.68). Increased tyrosine aminotransferase induction in the presence of inhibitors ('superinduction') was intensively studied by Tomkins in the late 1960s (Tomkins *et al.*, 1969). Recent work (Diesterhaft *et al.*, 1980) has established that the normal decay of tyrosine aminotransferase mRNA after induction was unchanged in the presence of actinomycin D, and it is suggested (see Diesterhaft *et al.*, 1980) that 'superinduction' may involve stabilization of the enzyme against degradation. The actions of Be²⁺ on the induction by dexamethasone of ornithine decarboxylase and of tyrosine aminotransferase leads to 'superinduction'. We suggest that following the induction of the enzyme under consideration there is a corresponding induction of a specific degrading enzyme. Thus, depending on the times at which these two events take place and assuming that both inductions are inhibited by Be²⁺, if Be²⁺ is given after induction of the first enzyme the so-called 'superinduction' will be evident.
Table 1. The effects of Be\textsuperscript{2+} on the induction of enzymes in rat liver

Be\textsuperscript{2+} (BeSO\textsubscript{4}: sulphosalicylic acid 1:1, 40 \textmu mol Be\textsuperscript{2+}/kg) was given intravenously 3\* or 18 h before induction. Dexamethasone-treated (0.5 mg/kg intraperitoneally) or partially hepatectomized rats were killed after 3 h. Enzyme units per g at 37\,^\circ\text{C}. Means + S.E.M. (no. of rats).

<table>
<thead>
<tr>
<th>Enzyme activities</th>
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<td><strong>A Intact rats</strong></td>
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<tr>
<td>Uninduced</td>
<td>0.1 ± 0.13 (3)</td>
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<tr>
<td>Uninduced + Be\textsuperscript{2+}</td>
<td>0.5 ± 0.13 (3)</td>
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<tr>
<td>Induced</td>
<td>5.0 ± 0.55 (4)</td>
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<tr>
<td>Induced + Be\textsuperscript{2+*}</td>
<td>2.0 ± 0.09 (4)</td>
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<tr>
<td>Induced + Be\textsuperscript{2+}</td>
<td>1.0,--1.2</td>
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<td><strong>B Surgically-treated rats</strong></td>
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<tr>
<td>Sham-operated</td>
<td>0.6 ± 0.18 (3)</td>
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<tr>
<td>Sham-operated + Be\textsuperscript{2+}</td>
<td>1.1 ± 0.29 (3)</td>
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<tr>
<td>Partially-hepatectomized</td>
<td>1.7 ± 0.46 (3)</td>
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<tr>
<td>Partially-hepatectomized + Be\textsuperscript{2+}</td>
<td>3.5 ± 0.44 (3)</td>
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<td><strong>C Intact rats</strong></td>
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<tr>
<td>Uninduced</td>
<td>9.3 ± 1.9 (5)</td>
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<tr>
<td>Uninduced + Be\textsuperscript{2+}</td>
<td>24.4 ± 3.7 (3)</td>
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<tr>
<td>Induced</td>
<td>35.7 ± 4.3 (4)</td>
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<td>Induced + Be\textsuperscript{2+}</td>
<td>41.0 ± 3.6 (6)</td>
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<td><strong>D Adrenalectomized rats</strong></td>
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<tr>
<td>Uninduced</td>
<td>9.1 ± 1.0 (3)</td>
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<tr>
<td>Uninduced + Be\textsuperscript{2+}</td>
<td>15.6 ± 1.4 (3)</td>
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**Transcription of ribonucleoprotein particles**

Properties of the ribonucleoprotein particles confirmed those described by Louis and Sekeris (1976). Giving \[^{32}\text{P}]\text{Pi} overnight, with short exposures to \[^{3}\text{H}]\text{Orotic acid immediately before death, showed that rapidly labelled RNA was differentially extracted in ribonucleoprotein particles with isosmolar solutions at pH 8.0. No histones were detected in the heterogeneous proteins from the particles.**

Early work by Witschi (1970) had shown protein synthesis by normal rats to be unaffected by Be\textsuperscript{2+}. Consistent with this, Be\textsuperscript{2+} did not affect the uptake of \[^{3}\text{H}]\text{Orotic acid into ribonucleoprotein particles from nuclei of uninduced rats. Dexamethasone produces undetectable effects on the uptake of \[^{3}\text{H}]\text{Orotic acid into total**
OD\textsubscript{260} (200 cpm)

- A
- B
- C
- D
- E
- F

Tube number
nuclear RNA. With the ribonucleoprotein particles isolated here, the specific radioactivity of their RNA was slightly increased (+11%, +40%) in dexamethasone-treated rats. Two peaks of 3H radioactivity were apparent over the ribonucleoprotein (Fig. 1) with an indication that it was incorporation into lighter material which was promoted by dexamethasone (Fig. 1B; cf. 1A). This was also true of ribonucleoprotein particles from partially hepatectomized rats (Fig. 1C). Be2+ (40 μmol/kg) reduced the uptake of [3H]orotic acid by the particles from dexamethasone-treated rats by 50%, and by those from partially hepatectomized rats (in S phase) by 30%. The reduction was most evident in the material in the lighter fractions of the ribonucleoprotein particles (Fig. 1D, 1E). With 3 h exposure to 120 μmol Be2+/kg, coincident with the administration of the dexamethasone, the uptake of orotic acid into the lighter material virtually disappeared (Fig. 1F).

Further analysis of RNA from the particles was precluded by RNAase activity in rat liver, which is almost impossible to prevent (Heinrich et al., 1978).

The uptake of [32P]Pi into proteins of the ribonucleoprotein particles in the latter experiment, after the removal of nucleic acids in hot trichloroacetic acid, was increased 50% by dexamethasone. This increase vanished with the proteins from the Be2+-treated animals.

Earlier experiments (Ord & Stocken, unpublished) had shown that 32P specific radioactivity of the total 0.35 M NaCl-extractable non-histone proteins in nuclei from the livers of normal or induced rats was unaffected by Be2+ after RNA and histones had been removed from the preparations by hydroxyapatite or if the specific radioactivity

Fig. 1. Sucrose-gradient sedimentation profiles of rat-liver ribonucleoprotein particles. Ribonucleoprotein particles were isolated and sedimented through 15-30% sucrose gradients (15-17 h, SW 27 rotor, 27 000 rpm; Louis & Sekeris, 1976). 1-ml fractions were collected. The material was lighter with increasing tube number. [3H]orotic acid was given to the rats intramuscularly 15 min before death. 3-6 rats per group.
A. Ribonucleoprotein particles from control, uninduced rat-liver nuclei.
B. Particles from rats which had been given dexamethasone (0.5 mg/kg) 3 h before death.
C. Particles from rats which had been partially hepatectomized 21 h previously.
D. As B, except that the rats had received Be2+ (40 μmol/kg) 18 h before being given dexamethasone.
E. As C; Be2+ (40 μmol/kg) given 18 h before partial hepatectomy.
F. As B; Be2+ (120 μmol/kg) given 3 h before death.
of only alkali-labile Pi was determined. Rigorous analytical techniques will therefore be required to identify the non-histone protein(s) within the ribonucleoprotein particles whose phosphorylation, presumably by cytoplasmic protein kinase (Kaser et al., 1980), is promoted by dexamethasone and inhibited by Be²⁺.

There is of course the possibility that the effects of Be²⁺ on the inducing ability of dexamethasone in rat liver are also associated with diminished phosphorylation of the glucocorticoid carrier protein in the cytoplasm. This modification is believed to be essential for the binding of the steroid (Nielson et al., 1977) and thus the transport of the complex to the nucleus. We have not been able to establish that Be²⁺ interferes in this way.

Acknowledgements

We are grateful to Mr. M.R. Kaser for his assistance in the experimental work reported here.

References