Induction of hepatic and renal ornithine decarboxylase by cobalt and other metal ions in rats

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We previously showed that Cd^{2+} is able to induce hepatic and renal ornithine decarboxylase (ODC). In addition to Cd^{2+} , the administration of Co^{2+} and other metal ions such as Se^{2+} , Zn^{2+} and Cr^{2+} produced a significant increase of hepatic and/or renal ODC activity. Of the metal ions used in this study, Co^{2+} produced the greatest increase of ODC activity. The maximum increases in hepatic and renal ODC activity, to respectively 70 and 14 times the control values in male rats, were observed 6 h after the administration of Co^{2+} . A similar response was seen in the liver, but not in the kidney, of female rats. Thereafter, ODC activity gradually returned to control values in the liver, but it was profoundly decreased to 7% of the control value at 24 h in the kidney. The pretreatment of animals with either actinomycin D or cycloheximide almost completely blocked the Co^{2+} -mediated increase of ODC activity. Co^{2+} complexed with either cysteine or glutathione (GSH) failed to induce ODC. Depletion of hepatic GSH content by treatment of rats with diethyl maleate greatly enhanced the inducing effect of Co^{2+} on ODC. The inhibitors of ODC, 1,3-diaminopropane and α -difluoromethylornithine, were able to inhibit the induction of the enzyme, without affecting the induction of haem oxygenase by Co^{2+} . Methylglyoxal bis(guanyldrazone), an inhibitor of *S*-adenosylmethionine decarboxylase, significantly inhibited the Co^{2+} -mediated induction of both ODC and haem oxygenase. It is suggested that the inducing effects of Co^{2+} on ODC and haem oxygenase are brought about in a similar manner.

INTRODUCTION

Ornithine decarboxylase (ODC; EC 4.1.1.17) is the initial and rate-limiting enzyme in the biosynthesis of polyamines, which are involved in the control of cell growth and in the response to hormonal and toxic stimuli (Morris & Fillingame, 1974; Jänne *et al.*, 1978; Canellakis *et al.*, 1979; Russell, 1983; Tabor & Tabor, 1984).

We have described the ability of Cd^{2+} to induce hepatic and renal ODC in male rats (Yoshida *et al.*, 1984). There was a sex-related difference in the response to Cd^{2+} with respect to renal ODC induction in rats, male rats responding appreciably, but not female rats. Although a mechanism for the induction of hepatic and renal ODC by Cd^{2+} remained to be clarified, the early induction pattern of the enzyme by the metal ion seemed to parallel that of haem oxygenase associated with the decrease in cytochrome *P*-450 content in the liver (Krasny & Holbrook, 1977; Yoshida *et al.*, 1979).

The induction of haem oxygenase associated with the decrease in cytochrome *P*-450 is likely to be a common phenomenon seen after the acute administration of metal ions, including Cd^{2+} (Maines & Kappas, 1976, 1977). Among metal ions, Co^{2+} and Cd^{2+} are the most potent inducers of hepatic haem oxygenase (Maines & Kappas, 1974, 1976, 1977; Kikuchi *et al.*, 1982). Taking these facts into consideration, together with our previous findings on the induction of ODC by Cd^{2+} (Yoshida *et al.*, 1984), the present investigation was undertaken in order to examine the abilities of Co^{2+} and other metal ions to stimulate

hepatic and renal ODC. In addition, this study was also designed to examine whether there is an interrelation between the induction of ODC and haem oxygenase.

MATERIALS AND METHODS

Chemicals

DL-[1- ^{14}C]Ornithine (55 mCi/mmol) was purchased from Japan Radioisotope Association (Tokyo, Japan). NADPH, NADP $^{+}$, glucose 6-phosphate and glucose-6-phosphate dehydrogenase were from Kyowa Hakko Co. (Tokyo, Japan). Haemin was obtained from Sigma Chemical Co. DFMO was kindly given by Dr. W. J. Hudak, Merrell Dow Pharmaceuticals, Cincinnati, OH, U.S.A. All other chemicals were of the highest grade available commercially.

Animals and treatment

Male and female Wistar rats, weighing 180–200 g, were used throughout the study. Rats were injected subcutaneously with a single dose (250 $\mu\text{mol/kg}$ body wt., unless otherwise indicated) of CoCl_2 , or CoCl_2 in the same dose mixed with cysteine or glutathione in a 1:3 molar ratio. Other metal compounds studied were CdCl_2 (25 $\mu\text{mol/kg}$), Na_2SeO_3 (12.5 $\mu\text{mol/kg}$), ZnCl_2 (125 $\mu\text{mol/kg}$) and K_2CrO_4 (125 $\mu\text{mol/kg}$). Where indicated, rats were pretreated with diethyl maleate (0.6 ml/kg, intraperitoneally) 30 min before the administration of Co^{2+} . In some experiments, rats were injected intraperitoneally with 1,3-diaminopropane (150 mg/kg) or DFMO (500 mg/kg) just before and 3 h after the administration of Co^{2+} , or

Abbreviations used: ODC, ornithine decarboxylase; DFMO, α -difluoromethylornithine; MGBG, methylglyoxal bis(guanyldrazone).

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with MGBG (60 mg/kg) just before the administration of Co^{2+} . The animals were starved overnight before the injection of metal ions and were then killed at appropriate time periods, as indicated in the Figure and Tables. The control animals received an injection of 0.9% NaCl.

Tissue preparation

Livers were perfused *in situ* with 0.9% NaCl, excised, and homogenized (1:6, w/v) in 0.05 M-sodium/potassium phosphate buffer (pH 7.2) containing 1 mM-dithiothreitol, 0.1 mM-EDTA and 40 μM -pyridoxal phosphate. The homogenates were centrifuged at 9000 *g* for 20 min, and the resulting supernatant fractions were again ultracentrifuged at 105000 *g* for 60 min. The final supernatant fractions were used for the determination of ODC activity. The microsomal pellets were washed once and resuspended in buffer, and used for the determination of haem oxygenase activity. Kidneys were homogenized in the same buffer as described above, and the homogenates were centrifuged at 20000 *g* for 20 min. The resulting supernatant fractions were used for the determination of ODC activity.

Enzyme assays

ODC activity was determined by the method of Russell & Snyder (1968) as described previously (Yoshida *et al.*, 1984). Briefly, the reaction mixture, comprising 0.1 M-sodium phosphate buffer (pH 7.2), 2 mM-dithiothreitol, 0.2 mM-EDTA, 80 μM -pyridoxal phosphate, 0.25 mM-L-ornithine hydrochloride, 0.4 μCi of DL-[1- ^{14}C]ornithine and 0.1 ml of supernatant fraction, in a final volume of 0.5 ml, was incubated for 30 min at 37 °C. After incubation, the reaction was terminated by the addition of 0.5 ml of 2 M-citric acid and left for an additional 60 min while the liberated $^{14}\text{CO}_2$ was absorbed by filter paper saturated with β -phenethylamine, by using the procedure as described by Ichiyama *et al.* (1970). Microsomal haem oxygenase activity was determined by the method of Tenhunen *et al.* (1970), by using the 105000 *g* supernatant fractions obtained from control rats as the source of biliverdin reductase. Protein concentrations were determined by the method of Lowry *et al.* (1951) for microsomal fraction and by that of Gornall *et al.* (1949) for supernatant fractions from both liver and kidney.

RESULTS

Table 1 represents the effects of metal ions, including Cd^{2+} , as we have previously shown (Yoshida *et al.*, 1984), on hepatic and renal ODC activity. The injected doses and route of administration of the metal ions and the time of killing the animals, were chosen on the basis of data described by Maines & Kappas (1976), and from our previous results with Cd^{2+} (Yoshida *et al.*, 1984), respectively. In addition to Cd^{2+} , the administration of other metal ions, such as Se^{2+} , Cr^{2+} and Co^{2+} , significantly increased hepatic and renal ODC activity. The magnitude of the increase in these activities produced by these metal ions was about 16–32 times and 4–21 times the corresponding control values respectively. The treatment of animals with Zn^{2+} increased only renal ODC activity, about 6 times compared with controls. Among the metal ions, including Cd^{2+} , used in these experiments, Co^{2+} gave the most potent increase in ODC activity. From these results, together with the findings indicating

Table 1. Effects of metal ions on hepatic and renal ODC activity

Male rats were injected intraperitoneally with CdCl_2 (25 $\mu\text{mol/kg}$), Na_2SeO_3 (12.5 $\mu\text{mol/kg}$) or ZnCl_2 (125 $\mu\text{mol/kg}$), or subcutaneously with K_2CrO_4 (125 $\mu\text{mol/kg}$) or CoCl_2 (250 $\mu\text{mol/kg}$), 4 h before being killed for the determination of ODC activity. Values are means $\pm$ S.E.M. for three to four rats: * $P < 0.05$ compared with control (saline).

Treatment	ODC activity (pmol/30 min per mg of protein)	
	Liver	Kidney
Saline (intraperitoneal)	4.62 $\pm$ 0.64	28.9 $\pm$ 4.2
CdCl_2	43.4 $\pm$ 8.03*	239 $\pm$ 65*
Na_2SeO_3	72.9 $\pm$ 12.3*	105 $\pm$ 41*
ZnCl_2	9.56 $\pm$ 3.50	162 $\pm$ 68*
Saline (subcutaneous)	2.85 $\pm$ 0.57	12.4 $\pm$ 3.4
K_2CrO_4	57.6 $\pm$ 13.8*	46.5 $\pm$ 9.3*
CoCl_2	81.1 $\pm$ 14.5*	257 $\pm$ 61*

Fig. 1. Time course of the effect of Co^{2+} on hepatic and renal ODC activity

Male rats were injected with CoCl_2 (250 $\mu\text{mol/kg}$, subcutaneously) and killed at the times indicated for the determinations of hepatic (○) and renal (●) ODC activity. Results are means $\pm$ S.E.M. (bars) for three rats.

that Co^{2+} is a potent inducer of hepatic haem oxygenase, as described by Maines & Kappas (1976), we chose Co^{2+} as a typical metal ion for investigating the increased pattern of ODC activity, and a possible mechanism for the increase of enzyme activity in the following experiments.

Fig. 1 shows the time course of the increases in hepatic and renal ODC activity after subcutaneous administration of CoCl_2 at a dose of 250 $\mu\text{mol/kg}$. The administration of Co^{2+} significantly increased hepatic ODC activity during 4–24 h after its administration. The maximum

Table 2. Effect of CoCl₂ on hepatic and renal ODC activity in male and female rats

Male and female rats were treated subcutaneously with CoCl₂ and killed 6 h later for the determination of hepatic and renal ODC activity. Each value is the mean $\pm$ S.E.M. for three rats: **P* < 0.05 compared with control (saline).

Treatment	ODC activity (pmol/30 min per mg of protein)			
	Liver		Kidney	
	Male	Female	Male	Female
Saline	1.30 $\pm$ 0.15	2.63 $\pm$ 0.63	41.5 $\pm$ 24.1	33.0 $\pm$ 13.6
CoCl ₂	53.1 $\pm$ 17.3*	52.0 $\pm$ 19.4*	276 $\pm$ 96*	35.7 $\pm$ 10.3

increase in hepatic ODC activity seen at 6 h after the administration was to approx. 70 times the control values under the experimental conditions. The administration of Co²⁺ also resulted in the profound increase in renal ODC activity during 4–8 h, with maximum increase of 14 times the control values at 6 h after the treatment. The changed pattern of renal ODC activity differed somewhat from that of hepatic ODC activity, namely that the sharp increase and decline in renal ODC activity was observed during the early time periods, and the enzyme activity was decreased to only 7% of the control value when determined at 24 h after treatment with Co²⁺. However, the decreased renal ODC activity returned to control values at 48 h. The results thus indicated that Co²⁺ is able to produce the increase in both hepatic and renal ODC activity during the early time periods, but to decrease renal enzyme activity at the later time period after its administration. From these results, we chose the 6 h time period after Co²⁺ administration in the following experiments. Co²⁺ increased hepatic and renal ODC activity in a dose-dependent manner. The administration of Co²⁺ at a dose of 125 μ mol/kg was able to increase hepatic and renal ODC activity, and a dose of 500 μ mol/kg greatly increased both enzyme activities, to 3 times the values obtained with the dose of 250 μ mol/kg which was used routinely in this study (results not shown).

We previously described the lack of renal ODC induction in female rats after the administration of Cd²⁺ (Yoshida *et al.*, 1984). Thus experiments were also done to find whether there is such a lack of response of renal ODC in female rats with Co²⁺ also. As shown in Table 2, the treatment of female rats with Co²⁺ resulted in a remarkable increase in hepatic ODC, as seen in male rats. However, Co²⁺ did not show any effect on renal ODC of female rats when determined under the same experimental conditions. It is therefore likely that the response of female rat kidney to metal ions is different from that of male rat kidney with respect to ODC induction.

The effect on ODC activity of Co²⁺ *in vitro* was also examined. The addition of Co²⁺ to the reaction mixture at concentrations used (up to 0.1 mM) did not show any stimulatory or inhibitory effect on ODC activity, but inhibited at higher concentrations (above 1 mM) (results not shown). The results indicate that the early increase in hepatic and renal ODC, and the later decrease in renal enzyme activity seen after the administration of Co²⁺ *in vivo*, did not result from direct activation and inhibition of the preformed enzyme respectively.

The data shown in Table 3 strongly suggest that the increase in hepatic and renal ODC found *in vivo* after the

Table 3. Effects of pretreatment of rats with actinomycin D and cycloheximide on the Co²⁺-mediated increase in hepatic and renal ODC activity

Male rats were pretreated intraperitoneally with actinomycin D (2 mg/kg) and cycloheximide (5 mg/kg) 1 h before administration of CoCl₂, and killed 6 h later for the determination of ODC activity. Each value is the mean $\pm$ S.E.M. for three rats: **P* < 0.05 (different from control); †*P* < 0.05 (different from CoCl₂).

Treatment	ODC activity (pmol/30 min per mg of protein)	
	Liver	Kidney
Saline	5.22 $\pm$ 1.36	53.0 $\pm$ 19.6
Actinomycin D	3.02 $\pm$ 1.30	23.5 $\pm$ 8.7
Cycloheximide	1.22 $\pm$ 0.29*	1.04 $\pm$ 0.27
CoCl ₂	225 $\pm$ 39*	354 $\pm$ 108*
Actinomycin D + CoCl ₂	5.31 $\pm$ 1.55†	24.4 $\pm$ 7.3†
Cycloheximide + CoCl ₂	4.31 $\pm$ 3.24†	2.02 $\pm$ 2.02†

administration of Co²⁺ could be due to the increased synthesis of new enzyme protein. The pretreatment of rats with either actinomycin D or cycloheximide, well-known inhibitors of protein synthesis, resulted in a complete inhibition of the Co²⁺-mediated increase in hepatic and renal ODC. Thus it would be reasonable to suggest that Co²⁺ is a potent inducer of hepatic and renal ODC.

The inducing effect of Co²⁺ on hepatic haem oxygenase has been well documented and extensively studied (Maines & Kappas, 1974, 1976). The early patterns of Co²⁺-mediated induction of haem oxygenase (Maines & Kappas, 1974) and ODC, as shown in the present study, are very similar. Because of the important role of polyamines in regulating protein synthesis, the experiments were carried out to examine whether there is any similarity between the induction of haem oxygenase and ODC. Maines & Kappas (1976) have shown that the induction of haem oxygenase by Co²⁺ was cancelled when Co²⁺ was administered as complexes with cysteine and glutathione. This finding prompted us to examine the effects of cobalt complexes on hepatic ODC and haem oxygenase activity. The data shown in Table 4 indicate that Co²⁺ complexed with either cysteine or glutathione was ineffective in inducing hepatic haem oxygenase. The results thus confirmed the previous findings described by Maines & Kappas (1976). Likewise, cobalt complexes were unable to induce hepatic ODC under the same

Table 4. Effects of cobalt complexes on hepatic ODC and haem oxygenase activities

Rats were injected with CoCl_2 (250 $\mu\text{mol/kg}$, subcutaneously) or with cobalt complexed with either cysteine or glutathione as described in the text, and killed 6 h later for the determinations of hepatic ODC and haem oxygenase activities. Neither cysteine nor glutathione alone showed any effect on the basal enzyme activities. Values are means $\pm$ S.E.M. for three rats: * $P < 0.05$ compared with controls.

Treatment	ODC activity (pmol/30 min per mg of protein)	Haem oxygenase activity (nmol/h per mg of protein)
Control	2.45 $\pm$ 0.42	2.16 $\pm$ 0.09
CoCl_2	215 $\pm$ 60.5*	13.7 $\pm$ 1.24*
CoCl_2 + cysteine	2.48 $\pm$ 0.48	2.06 $\pm$ 0.30
CoCl_2 + glutathione	11.5 $\pm$ 2.90	2.59 $\pm$ 0.04

Table 5. Influence of pretreatment with diethyl maleate on the effect of Co^{2+} on hepatic ODC and haem oxygenase activities

One group of rats was injected intraperitoneally with diethyl maleate (0.6 ml/kg) 30 min before the injection of CoCl_2 (125 $\mu\text{mol/kg}$). Other groups were injected with diethyl maleate or CoCl_2 at the above dosages. The animals were killed 6 h after administration of CoCl_2 , for the determinations of hepatic ODC and haem oxygenase activities. Values are means $\pm$ S.E.M. for three rats: * $P < 0.05$ (different from control); † $P < 0.05$ (different from either diethyl maleate or CoCl_2).

Treatment	ODC activity (pmol/30 min per mg of protein)	Haem oxygenase activity (nmol/h per mg of protein)
Control	4.56 $\pm$ 0.36	2.48 $\pm$ 0.14
CoCl_2	82.7 $\pm$ 28.7*	10.6 $\pm$ 3.11*
Diethyl maleate	63.6 $\pm$ 19.4*	6.38 $\pm$ 1.90*
Diethyl maleate + CoCl_2	316 $\pm$ 57.8†	15.6 $\pm$ 2.35†

experimental conditions. The administration of cysteine or glutathione alone did not produce any increase of ODC activity or of haem oxygenase activity. The results suggest that the free ionic form of cobalt would be necessary to induce both haem oxygenase and ODC, and that the induction of both enzymes by the metal ion might take place in a similar manner.

Since a thiol group seems to be directly or indirectly involved in the inducibilities of haem oxygenase and ODC by Co^{2+} , we examined the effect of diethyl maleate, which profoundly depletes hepatic glutathione (Boylard & Chausseaud, 1970), on the Co^{2+} -mediated induction of haem oxygenase and ODC. As shown in Table 5, the administration of diethyl maleate itself evoked a significant increase of hepatic haem oxygenase activity, as reported by Burk & Correia (1979). In addition, diethyl maleate also produced a marked increase in ODC activity. This is the first report of the increase in hepatic

Table 6. Effects of 1,3-diaminopropane (DAP), DFMO and MGBG on the Co^{2+} -mediated induction of hepatic ODC and haem oxygenase

Rats were injected intraperitoneally with DAP (150 mg/kg) or DFMO (500 mg/kg), just before and 3 h after, or with MGBG (60 mg/kg) just before, the administration of CoCl_2 , and killed 6 h after the CoCl_2 administration for the determinations of hepatic ODC and haem oxygenase activities. DAP, DFMO and MGBG alone did not significantly alter the basal ODC and haem oxygenase activities. Values are means $\pm$ S.E.M. for three rats: * $P < 0.05$ (different from the control); † $P < 0.05$ (different from CoCl_2).

Treatment	ODC activity (pmol/30 min per mg of protein)	Haem oxygenase activity (nmol/h per mg of protein)
Control	2.87 $\pm$ 0.01	1.91 $\pm$ 0.56*
CoCl_2	120 $\pm$ 22.2*	9.26 $\pm$ 1.28*
DAP + CoCl_2	16.8 $\pm$ 10.2*†	7.19 $\pm$ 0.37*
DFMO + CoCl_2	14.4 $\pm$ 2.47*†	11.8 $\pm$ 2.99*
MGBG + CoCl_2	2.11 $\pm$ 0.16†	2.39 $\pm$ 0.73†

ODC by diethyl maleate, since no previous report has so far been found. The effects of both Co^{2+} and diethyl maleate on the increase in haem oxygenase activity were additive, whereas those on ODC activity showed strong synergy. The results suggest that the inducibility of hepatic ODC by Co^{2+} could be more extensively dependent on hepatic glutathione content than is that of haem oxygenase. Since there was enhancement of the induction of haem oxygenase by Co^{2+} in diethyl maleate-treated rats at the later time period (Maines & Kappas, 1976), the data obtained here and in the preceding experiments further imply that the induction of both haem oxygenase and ODC by Co^{2+} could be brought about in a similar manner.

In order to ascertain whether there is an interrelation between the induction of both haem oxygenase and ODC, the experiments with specific inhibitors of ODC, i.e. 1,3-diaminopropane (Piik *et al.*, 1978) and DFMO (Metcalfe *et al.*, 1978), or with the inhibitor of S-adenosylmethionine decarboxylase, MGBG (Williams-Ashman & Schenone, 1972), were conducted. As shown in Table 6, the simultaneous administration of 1,3-diaminopropane, DFMO or MGBG with Co^{2+} inhibited the metal-mediated induction of hepatic ODC. However, neither 1,3-diaminopropane nor DFMO showed any inhibitory effect on Co^{2+} -mediated induction of hepatic haem oxygenase. These findings indicate that the inducing effects of Co^{2+} on haem oxygenase and ODC could be discriminated by administering the inhibitors of the latter enzyme. Interestingly, however, MGBG was also able to inhibit the Co^{2+} -mediated induction of hepatic haem oxygenase. Although MGBG alone had no effect on the basal haem oxygenase activity, it significantly blocked induction of the enzyme by Co^{2+} . Thus the data shown in Table 6 indicate that MGBG is an effective inhibitor of Co^{2+} -mediated induction of haem oxygenase and ODC. The findings imply that the induction of haem oxygenase and ODC by Co^{2+} could be modulated by inhibiting polyamine synthesis at a step at or beyond S-adenosylmethionine decarboxylase.

DISCUSSION

The present investigation has revealed that Co^{2+} and other metal ions, in addition to Cd^{2+} as reported previously (Yoshida *et al.*, 1984), are able to increase hepatic and/or renal ODC activity. It appeared that Co^{2+} produced the most potent stimulation of hepatic and renal ODC activity among the metal ions (including Cd^{2+}) studied in this experiment (Table 1).

Time-course studies of the effect of Co^{2+} on hepatic and renal ODC activity indicated that the increase in hepatic enzyme activity was rapid and sustained for up to 24 h, and that there was a biphasic effect, early stimulation and later inhibition, on renal enzyme activity (Fig. 1).

The fact that the increase of hepatic and renal ODC activity evoked by Co^{2+} was almost completely blocked by pretreatment of rats with either actinomycin D or cycloheximide (Table 3) indicates that the increased synthesis of new enzyme protein could be brought about by the metal-ion administration, as shown previously with Cd^{2+} (Yoshida *et al.*, 1984). Therefore Cd^{2+} , Co^{2+} and other metal ions used in this study could act as inducers of ODC. It is unclear, however, at present whether this induction of hepatic and renal ODC by the acute administrations of Co^{2+} and other metal ions could result from their cytotoxic effects on these tissues. The lack of the effect of Co^{2+} , as well as Cd^{2+} (Yoshida *et al.*, 1984), on renal ODC in female rats (Table 2) suggests that the enzyme induction is not simply the result of a cytotoxic effect of the metal ions. Rather, there might be a yet unknown regulatory mechanism for ODC induction that is responsive to metal ions.

Maines & Kappas (1976) have shown that haem oxygenase was not induced by cobalt complexed with either cysteine or glutathione and that the enzyme induction was enhanced by the metal ion in diethyl maleate-pretreated rats. The present study has also shown similar results for the induction of ODC and of haem oxygenase, except for the additive increase in the latter enzyme activity in diethyl maleate-pretreated rats (Tables 4 and 5). On the basis of these facts, it is suggested that the induction of ODC by Co^{2+} could take place in a similar manner to that of haem oxygenase. Accordingly, as extensive studies on haem oxygenase induction by Maines & Kappas (1976) have shown, the bivalent ionic form of cobalt might be required for ODC induction as well. These results alternatively suggest that there might exist a cellular component(s) which has a metal-ion-binding or complexing site and which is directly or indirectly involved in the regulation of haem oxygenase and ODC synthesis.

Experiments with the inhibitors of ODC, 1,3-diaminopropane, an indirect inhibitor (Piik *et al.*, 1978), and DFMO, a highly specific and irreversible inhibitor (Metcalf *et al.*, 1978), imply that the induction of haem oxygenase and ODC could take place by independent processes, because neither inhibitor blocked the Co^{2+} -

mediated induction of the former enzyme (Table 6). It thus appears that the increased ODC activity induced by Co^{2+} , which may lead to an increased rate of polyamine synthesis, is not required for the induction of haem oxygenase by the metal ion.

However, MGBG, an inhibitor of *S*-adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972), almost completely inhibited Co^{2+} -mediated induction of haem oxygenase and ODC (Table 6). It is not clear at present how MGBG could inhibit the induction of haem oxygenase and ODC evoked by Co^{2+} .

Though the mechanism by which Co^{2+} and other metal ions induce ODC and haem oxygenase is not clear at present, this investigation could provide information on biochemical effects of metal ions concerned with polyamine synthesis.

REFERENCES

- Boyland, E. & Chausseaud, L. F. (1970) *Biochem. Pharmacol.* **19**, 1526–1529
- Burk, R. F. & Correia, M. A. (1979) *Res. Commun. Chem. Pathol. Pharmacol.* **24**, 205–207
- Canellakis, E. S., Viceps-Madore, D., Kyriakidis, D. A. & Heller, J. S. (1979) *Curr. Top. Cell. Regul.* **15**, 155–202
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766
- Ichiyama, A., Nakamura, S., Nishizuka, Y. & Hayaishi, O. (1970) *J. Biol. Chem.* **245**, 1699–1709
- Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293
- Kikuchi, G., Yoshida, T. & Ishizawa, S. (1982) *Adv. Pharmacol. Ther., Proc. Int. Congr. Pharmacol.* **8th** **5**, 121–130
- Krasny, D. R. & Holbrook, D. J. (1977) *Mol. Pharmacol.* **13**, 759–765
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maines, M. D. & Kappas, A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4293–4297
- Maines, M. D. & Kappas, A. (1976) *Biochem. J.* **154**, 125–131
- Maines, M. D. & Kappas, A. (1977) *Science* **198**, 1215–1221
- Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P. & Vever, J. P. (1978) *J. Am. Chem. Soc.* **100**, 2551–2553
- Morris, D. R. & Fillingame, R. H. (1974) *Annu. Rev. Biochem.* **43**, 303–325
- Piik, K., Pösö, H. & Jänne, J. (1978) *FEBS Lett.* **89**, 307–312
- Russell, D. H. (1983) *Adv. Enzyme Regul.* **21**, 201–222
- Russell, D. H. & Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420–1427
- Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790
- Tenhunen, R., Marver, H. S. & Schmid, R. (1970) *J. Lab. Clin. Med.* **75**, 410–420
- Williams-Ashman, H. G. & Schenone, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 288–295
- Yoshida, T., Suzuki, Y. & Hashimoto, Y. (1979) *Toxicol. Lett.* **4**, 97–102
- Yoshida, T., Numazawa, S. & Kuroiwa, Y. (1984) *Biochem. Biophys. Res. Commun.* **125**, 882–887