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HORMONAL REGULATION OF HEME OXYGENASE INDUCTION IN AVIAN HEPATOCYTE CULTURE

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Abstract—The effects of various hormones were examined on the induction of heme oxygenase in monolayer cultures in chick embryo hepatocytes maintained in a chemically defined medium. Addition of insulin to the cultured cells markedly suppressed the activity of basal as well as Co^{2+}-induced heme oxygenase. Treatment of cells with hydrocortisone also suppressed the basal enzyme activity, while the Co^{2+}-induced enzyme activity was enhanced slightly. In contrast, triiodothyronine addition to the culture caused a slight increase of both uninduced and induced levels of the enzyme. This stimulatory effect of triiodothyronine was enhanced significantly by prolonged incubation of cells (48–96 hr) in the serum-free medium. These findings indicate that heme oxygenase synthesis can be substantially altered by changing the hormonal environment of the hepatocytes. Furthermore, the induction of heme oxygenase by Co^{2+} was inhibited by glucagon, dibutyryl cAMP and theophylline in a dose-dependent manner, suggesting that the enzyme induction may also be controlled by changes in cAMP levels.

The microsomal enzyme heme oxygenase catalyzes the degradation of cellular heme to yield biliverdin IX_α [1]. The activity of this enzyme can be induced in a variety of tissues *in vivo* and in liver cells in culture by a number of inorganic and organic metals [2–6], certain toxins [7, 8] and by the substrate heme itself [9]. A marked induction† of hepatic heme oxygenase, as elicited by metals for example, causes a profound disturbance in heme metabolism in the liver and may result in enhanced degradation of microsomal cytochrome P-450, depression of cytochrome P-450-dependent drug oxidation activities and perturbations of δ-aminolevulinate synthase activity [10, 11]. The inducibility of heme oxygenase is known to be influenced by endocrine factors as has been shown in recent studies from this and other laboratories [12–16]. However, since most earlier studies were carried out in whole animals, it was not possible to definitely assign the role of a specific hormone in the control mechanism of heme oxygenase in view of the complex hormonal interactions which occur *in vivo*; for this reason the present study was undertaken to examine the influences which hormones exert on the induction of heme oxygenase in liver cells cultured in a chemically defined medium.

The results of this study indicate that triiodothyronine increased the induction of heme oxygenase mediated by the potent metal inducer of this enzyme, Co^{2+}, whereas insulin, dibutyryl cAMP and glucagon caused a decrease in basal as well as induced levels of heme oxygenase. Hydrocortisone caused a marked suppression of basal enzyme activity but an enhanced Co^{2+}-mediated induction of heme oxygenase.

† The term "induction" has been used in all instances where heme oxygenase activity has been increased substantially in liver cells by such treatments as metals, hemin and other organic chemicals because the increased enzyme activity is blocked by inhibitors of nucleic acid and protein synthesis and because such agents do not affect the enzyme *in vitro*.

EXPERIMENTAL PROCEDURES

Materials. For the culturing of chick embryo liver cells, a modified Ham's F-12 medium free of pyruvate, lipids and trace metals was used [17]. Hemin, crystalline bovine insulin, hydrocortisone, glucagon, dibutyryl cAMP, theophylline, triiodothyronine, L-isoproterenol, L-epinephrine, L-norepinephrine, $NADP^+$ and glucose-6-phosphate were purchased from the Sigma Chemical Co. Co^{2+}, used in the study as $CoCl_2$, was obtained from Fisher Scientific. Glucose-6-phosphate dehydrogenase was from Boehringer Mannheim.

Preparation of hepatocytes. Chick embryo hepatocytes were cultured as described earlier [17]. Seventeen-day-old chick embryos were perfused extensively through the heart to deplete the liver of red blood cells. Livers were then minced in a sterile mixture of 0.5% collagenase and 0.05% hyaluronidase for 10–15 min at room temperature. The disrupted cell suspension was centrifuged at 200 *g* for 60 sec. The supernatant fraction was discarded, 10 ml of NH_4/Tris/HCO_3 solution (0.13 M NH_4Cl, 0.017 M Tris base and 0.01 M $KHCO_3$, pH 7.65) was added to the pellet to hemolyze the contaminating red cells, and the mixture was centrifuged again at 200 *g* for 60 sec. The cell pellet was suspended in 75 vol. of the modified Ham's F-12 medium containing 5% fetal bovine serum. In some experiments in which triiodothyronine effects were examined, cells were prepared in a serum-free modified F-12 medium supplemented with bovine pancreas insulin (1 μg/ml). The preparation of 1 μg of insulin is equiv-

alent to 25 milli I.U. Experiments were started by the addition of 5 ml of cell suspension in dishes. After 24 hr, unattached cells were removed and the medium was replaced with fresh medium without serum. Chemical and hormone additions were made at this point. The cells were treated as described in the legends of the tables and figures. Triplicate cell culture dishes were used for each treatment.

Cell harvest and preparation of homogenates. At the end of incubation, the medium was removed by aspiration from the dishes and cells were washed with 5 ml of Earle's buffer salt solution. Cells were scraped from the dishes with a rubber policeman and centrifuged at 600 *g* for 5 min. The cell pellet was resuspended in 1 ml of 0.1 M potassium phosphate buffer, pH 7.4, and homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle. Each dish contained an average of 3–4 mg of cellular protein. Heme oxygenase activity was assayed in homogenates using rat liver supernatant fraction as a source of biliverdin reductase.

Preparation of rat liver supernatant fraction. Male adult rats weighing 170–180 g (Sprague–Dawley) were starved for 12 hr and thereafter killed by decapitation; livers were perfused with ice-cold 0.9% NaCl until clear of hemoglobin, homogenized in 3 vol. of 0.1 M potassium phosphate buffer, pH 7.4, and centrifuged at 9000 *g* for 20 min. The supernatant fraction was carefully removed and recentrifuged at 105,000 *g* for 60 min; the supernatant thus obtained was stored at −20° and used as the source of biliverdin reductase in the assay of heme oxygenase.

Heme oxygenase assay. The activity of heme oxygenase was determined in the whole cellular homogenates of cultured liver cells as described previously [6]. To the test sample of 0.3 ml (~3 mg protein/ml) was added 50 μl of 10 mg/ml of rat liver supernatant, an NADPH-generating system (comprised of 2 mM $MgCl_2$, 1.6 mM $NADP^+$, 0.8 mM glucose-6-phosphate, 0.1 unit of glucose-6-phosphate dehydrogenase), and 30 μM hemin in a final volume of 0.75 ml. The reference tube contained all ingredients except $NADP^+$ and glucose-6-phosphate dehydrogenase. The reaction was stopped after incubation in the dark at 37° for 15 min by immersing the tubes in ice. The concentration of bilirubin formed during the reaction was estimated by taking the difference spectrum of the sample and the reference cuvette between 400 and 560 nm in an Aminco DW2A spectrophotometer in the split beam mode and was calculated by using an extinction coefficient of 40 $mM^{-1}\,cm^{-1}$ between 464 and 530 nm [6].

Protein determination and statistical analysis. The concentration of protein in cellular homogenates was measured using bovine serum albumin as standard [18]. The statistical analysis of data was performed as described earlier [19].

RESULTS

Induction of heme oxygenase in isolated hepatocytes. In an earlier study we showed that Co^{2+} increases the activity of heme oxygenase in cultured chick embryo liver cells in a dose-dependent manner up to a metal concentration of 75 μM [6]. The time course of induction of heme oxygenase in response to 50 μM Co^{2+} in the culture medium, studied at various time intervals, is shown in Fig. 1. Heme oxygenase activity increased slowly and reached a maximum at 24 hr. At 36 and 48 hr the activity of heme oxygenase remained elevated provided Co^{2+} was present in the medium, but the enzyme activity declined if Co^{2+} was removed by changing the medium (results not shown). The results indicate that the presence of Co^{2+} is essential for the continued induction and maintenance of increased heme oxygenase levels, at least up to 48 hr in cultured hepatocytes.

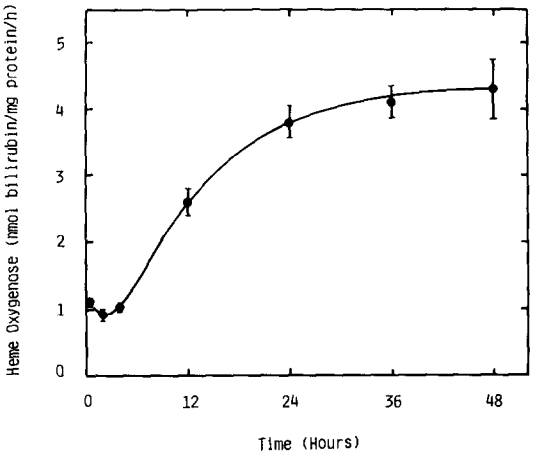

Fig. 1. Time course of Co^{2+} induction of heme oxygenase in cultured chick embryo liver cells. Hepatocytes were prepared and incubated in modified Ham's F-12 medium containing 5% fetal bovine serum. After 24 hr of incubation, the medium was discarded, and fresh medium without serum was added. $CoCl_2$ (50 μM) was added, and cells were incubated further for the time periods indicated on the abscissa. Heme oxygenase activity was determined as described in the text. Data are the mean ± S.E. of three determinations.

Effect of insulin on Co^{2+} induction of heme oxygenase. Since insulin has been shown to be required for both the growth of hepatocytes and for the induction of δ-aminolevulinate synthase by porphyrinogenic chemicals [17, 19], the effect of various insulin concentrations on the basal as well as on Co^{2+}-induced heme oxygenase activities in chick embryo liver cells was studied. Figure 2 shows the effect of insulin at various doses from 1 to 1000 ng/ml on the induction of heme oxygenase by Co^{2+}. At an insulin concentration of 1 ng/ml, the basal and Co^{2+}-induced activities of heme oxygenase were unchanged; however, at higher concentrations of insulin (10, 100 and 1000 ng/ml), there was a marked decrease in the activity of heme oxygenase in the control cells. The activity of heme oxygenase in cultures in which the enzyme had been earlier induced by Co^{2+}, also decreased by about 40% at 100 and 1000 ng/ml of insulin concentrations. The extent of suppression of heme oxygenase activity by insulin in cultured liver cells was greater in control than in Co^{2+}-treated cultures, thus showing that both

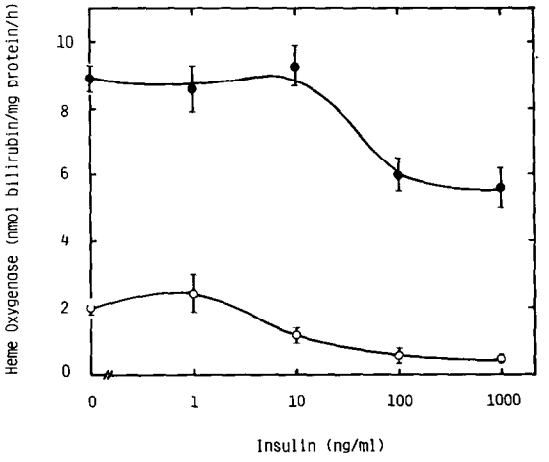

Fig. 2. Dose–response curve of the effect of insulin on Co^{2+} induction of heme oxygenase in cultured chick embryo liver cells. Cells were prepared and incubated as described in the text. After incubation for 24 hr, the medium was replaced with fresh medium without serum. The cells were treated with $CoCl_2$ (50 μM) and insulin at concentrations indicated on the abscissa. After 24 hr of incubation, heme oxygenase activity was determined as described in the text. Data are the mean values ± S.E. of at least three determinations. Key: (○——○) control; and (●——●) Co^{2+} treated.

basal as well as Co^{2+}-induced enzyme activities are modulated by insulin.

Effect of hydrocortisone on Co^{2+}-induction of heme oxygenase. In cultured chick embryo liver cells, it was shown earlier that addition of hydrocortisone and insulin increases porphyrin production by >30-fold in response to allylisopropylacetamide or steroids [17, 19]. The effect of hydrocortisone on the uninduced and the Co^{2+}-induced activity of heme oxygenase is shown in Fig. 3. Addition of low concentrations of hydrocortisone (10^{-9} M and 10^{-8} M) did not alter either the basal or the Co^{2+}-induced activities of heme oxygenase. Higher concentrations of hydrocortisone (10^{-7} M and 10^{-6} M), however, caused a marked suppression of the basal heme oxygenase level in the liver cells. In contrast, the addition of hydrocortisone at 10^{-7} and 10^{-6} M in Co^{2+}-treated cells increased the induction of heme oxygenase (~20%). The suppressive effect of hydrocortisone on uninduced heme oxygenase activity in cultured cells was analogous to the effect of this hormone observed on basal levels of the enzyme in adrenalectomized rats *in vivo* [14].

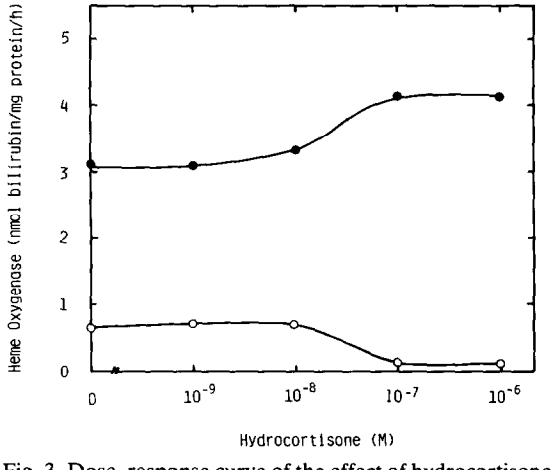

Fig. 3. Dose–response curve of the effect of hydrocortisone on Co^{2+} induction of heme oxygenase in cultured chick embryo liver cells. Cells were prepared and incubated as described in the text. After 24 hr of incubation, the medium was replaced with fresh medium without serum. The cells were treated with $CoCl_2$ (50 μM) and hydrocortisone at concentrations shown on the abscissa. After incubation for 24 hr, heme oxygenase activity was determined. Data are expressed as the mean of duplicate determinations. Key: (○——○) control; and (●——●) Co^{2+} treated.

Effect of triiodothyronine on heme oxygenase in isolated liver cells. Triiodothyronine in animals has been shown to influence cytochrome P-450 levels and cytochrome P-450-dependent drug metabolism [20–24]. Recently, it has been shown that hepatic heme oxygenase levels are increased significantly in response to triiodothyronine administration in hypothyroid rats [15]. This increased activity of heme oxygenase was associated with a concurrent decrease in hepatic cytochrome P-450 content. To determine whether chick embryo liver cells in culture would respond to triiodothyronine in a similar manner, heme oxygenase activities were determined after treating cells with various concentrations of triiodothyronine (Table 1). The basal activity of heme oxygenase increased by about 25–50% at 0.01, 0.1 and 1 μg/ml of triiodothyronine. The Co^{2+}-induction of heme oxygenase also showed a slight increase at all the concentrations of triiodothyronine. To determine if possible depletion of bound triiodothyronine from the liver cells by prolongation of the culture period might enhance the effect of the hormone on the induction process, we maintained the liver cells for 48, 72 and 96 hr, with changes of the fresh medium containing insulin (1 μg/ml) at each 24 hr, and then treated the cell cultures with triiodothyronine (1 μg/ml) for the last 24 hr before harvesting. Figure 4 shows the effect of triidothyronine treatment of liver cell cultures maintained for extended periods prior to the addition of the hormone and Co^{2+}. The uninduced activity of heme oxygenase was increased by the addition of triiodothyronine at all time intervals. The increase in heme oxygenase activity was greater after extended preincubation of cells without the hormone and at 96 hr was almost double ($P < 0.05$) that of untreated cultures. Similarly, Co^{2+} inducibility of heme oxygenase activity increased as a function of lengthened preincubation periods. The addition of triiodothyronine to the Co^{2+}-treated cultures increased the induction response of heme oxygenase activity markedly at all time points.

Effects of combined treatment with insulin, hydrocortisone and triiodothyronine on heme oxygenase. An earlier study from our laboratory showed that combined treatment of cultured chick embryo hepatocytes with insulin, hydrocortisone and triiodothyronine greatly enhances the induction response of δ-aminolevulinate synthase as compared with the response using insulin alone, after treatment with the

Table 1. Effect of triiodothyronine on the basal and Co^{2+}-mediated heme oxygenase induction in cultured chick embryo liver cells*

Treatment	Triiodothyronine conc (μg/ml)	Heme oxygenase (nmoles bilirubin/mg protein/hr)
Control	0	1.85 ± 0.05
	0.01	2.64 ± 0.15†
	0.1	2.88 ± 0.35‡
	1	2.72 ± 0.22‡
	10	2-15 ± 0.17§
Co^{2+}	0	7.31 ± 0.64
	0.01	8.89 ± 0.48‖
	0.1	8.01 ± 0.32‖
	1	8.25 ± 0.42‖
	10	8.19 ± 0.57‖

* Liver cells from chick embryos were prepared and incubated for 24 hr in modified Ham's F-12 medium supplemented with 5% fetal bovine serum. The medium was then replaced with fresh medium containing different concentrations of triiodothyronine. Co^{2+} (final concentration 50 μM) was also added in appropriate cultures at this time and, after 24 hr of further incubation, heme oxygenase activity was determined in homogenates as described in the text. Data represent the mean ± S.E. of at least three dishes.

† $P < 0.01$.

‡ $P < 0.05$.

§ Not significant as compared to control.

‖ Not significant as compared to Co^{2+}-treated.

porphyrinogenic chemical, allylisopropylacetamide [17]; similar effects have been observed in response to steroid inducers of the enzyme [19]. To examine the effects of these hormones on heme oxygenase, we studied the influence of combined treatment with insulin, hydrocortisone and triiodothyronine on the induction of heme oxygenase in response to Co^{2+} (Table 2) or hemin, the substrate as well as the known inducer of the enzyme [9] (Table 3). Insulin (1 μg/ml), hydrocortisone (0.05 μg/ml) and triiodothyronine (1 μg/ml) when added together decreased basal heme oxygenase activity about 60% compared with untreated controls (Table 2). The same combination of hormones, however, did not affect the Co^{2+}-induced levels of the enzyme. Table 3 shows the effects of insulin, hydrocortisone and triiodothyronine treatment on hemin-induced levels of heme oxygenase. Treatment of cultures with insulin or with the combination of insulin, hydrocortisone and triiodothyronine caused a 40% decline in hemin induction of heme oxygenase, while hydrocortisone alone did not appreciably affect hemin-mediated induction of the enzyme. The addition of triiodothyronine together with hemin slightly elevated the level of heme oxygenase (Table 3). Insulin alone at a concentration of 1 μg/ml caused a decrease (40%) in hemin-mediated heme oxygenase induction. A similar effect of insulin was also observed in the case of Co^{2+} induction of heme oxygenase (Fig. 2). This effect of insulin in decreasing Co^{2+} and hemin-mediated heme oxygenase induction was distinct from the permissive role that this hormone plays in the induction of δ-aminolevulinate synthase in the same cell type [17, 19, 25].

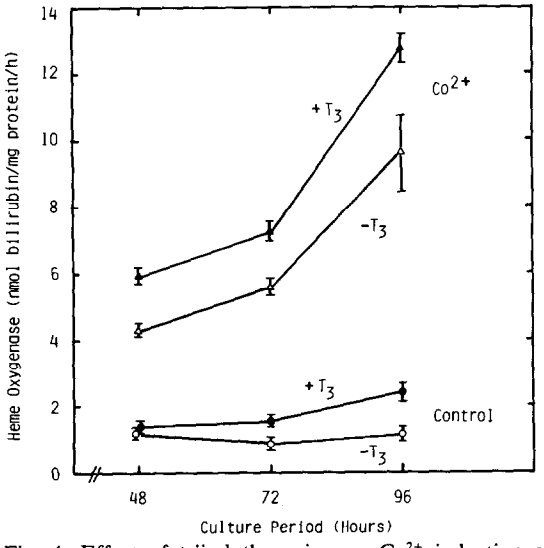

Fig. 4. Effect of triiodothyronine on Co^{2+} induction of heme oxygenase in chick embryo liver cells with extended periods of incubation. Cells were prepared and incubated in Ham's F-12 medium containing insulin (1 μg/ml). After every 24 hr of incubation, the medium was discarded and fresh medium containing insulin (1 μg/ml) was added. The cells were treated with $CoCl_2$ (50 μM) and triiodothyronine (1 μg/ml) for the last 24-hr period, and then heme oxygenase activity was determined as described in the text. Data are expressed as the mean ± S.E. of at least three determinations. Key: (○——○) control; (●——●) triiodothyronine; (△——△) Co^{2+}; (▲——▲) Co^{2+} plus triiodothyronine.

Effects of dibutyryl cAMP, glucagon, theophylline, epinephrine, norepinephrine and isoproterenol on the induction of heme oxygenase. Hepatic glucose metabolism *in vivo* is under the influence of a variety of hormones, and glycogenolysis is stimulated by the release of glucagon and epinephrine [26, 27]. Since

Table 2. Influence of insulin, hydrocortisone and triiodothyronine on the basal and Co^{2+}-mediated heme oxygenase induction in cultured chick embryo liver cells*

Additions	Heme oxygenase (nmoles bilirubin/mg protein/hr)
None	1.88 ± 0.07
Insulin (1 μg/ml) + hydrocortisone (0.05 μg/ml) + triiodothyronine (1 μg/ml)	0.77 ± 0.03†
Co^{2+} (50 μM)	5.60 ± 0.04
Insulin (1 μg/ml) + hydrocortisone (0.05 μg/ml) + triiodothyronine (1 μg/ml) + Co^{2+} (50 μM)	6.53 ± 0.32‡

* Liver cells were prepared and incubated in modified Ham's F-12 medium containing 5% fetal bovine serum as described in the text. After 24 hr of incubation, the medium was discarded, cultures were rinsed with Earle's buffer solution, and fresh medium without serum was added. Chemicals and hormones were also added at this time. Cells were further incubated for 24 hr, and heme oxygenase activity was determined in homogenates as described in the text. Data are expressed as the means ± S.E. of three determinations.

† $P < 0.01$.

‡ $P < 0.05$.

hepatic δ-aminolevulinate synthase activity and cytochrome P-450 were induced in response to glucagon addition in isolated liver cells [28], the effects of such hormones, which primarily affect glucose metabolism, on the induction of heme oxygenase were studied. Figure 5A shows the effect of glucagon concentration on the basal and Co^{2+}-induced levels of heme oxygenase. Addition of glucagon from 5×10^{-7} M to 5×10^{-4} M did not affect basal levels of the enzyme greatly, while Co^{2+} induction of heme oxygenase was inhibited markedly (40%) at 5×10^{-5} M and 5×10^{-4} M concentrations. Since addition of glucagon to hepatocytes is known to cause increased synthesis of cAMP [26, 27], the direct effect of dibutyryl cAMP addition was studied on heme oxygenase induction (Fig. 5B). Addition of dibutyryl cAMP to the incubation medium suppressed Co^{2+}-mediated induction of heme oxygenase in a dose-dependent manner. The inhibition was almost complete at the maximum concentration of 5×10^{-3} M of dibutyryl cAMP employed. These findings suggest that glucagon-stimulated glycogenolysis, a cAMP-mediated process, inhibits Co^{2+}-induced heme oxygenase in cultured hepatocytes. The inhibitory effects of glucagon and of dibutyryl cAMP on the heme oxygenase induction process were mimicked by the addition of theophylline (Fig. 5C), epinephrine, norepinephrine and isoproterenol (Fig. 6A–C), agents known to cause an increase in endogenous cAMP concentration. The suppressive effect observed with most agents was dose-dependent but, in comparable concentrations, isoproterenol was less effective than epinephrine or norepinephrine (Fig. 6A–C).

DISCUSSION

The present study demonstrates that a variety of hormones can alter the basal levels of activity as well as the induction response of heme oxygenase

Table 3. Effects of insulin, hydrocortisone and triiodothyronine on hemin-mediated induction of heme oxygenase in cultured chick embryo liver cells*

Additions	Heme oxygenase (nmoles bilirubin/mg protein/hr)
None	1.88 ± 0.07
Hemin (100 μM)	5.58 ± 0.41
Insulin (1 μg/ml) + hemin (100 μM)	3.46 ± 0.35†
Hydrocortisone (0.05 μg/ml) + hemin (100 μM)	5.08 ± 0.08‡
Triiodothyrone (1 μg/ml) + hemin (100 μM)	6.43 ± 0.38‡
Insulin (1 μg/ml) + hydrocortisone (0.05 μg/ml) + triiodothyronine (1 μg/ml) + hemin (100 μM)	3.32 ± 0.11§

* Cells were prepared and cultivated as described in the text. The medium was replaced with a fresh medium without serum after 24 hr of incubation, and hormones were added at this time. After incubation for 24 hr, cells were collected, washed and homogenized. Heme oxygenase activity in whole homogenates was determined as described in the text. Data represent the mean ± S.E. of three determinations.

† $P < 0.02$, compared to hemin treatment.

‡ Not significant.

§ $P < 0.01$.

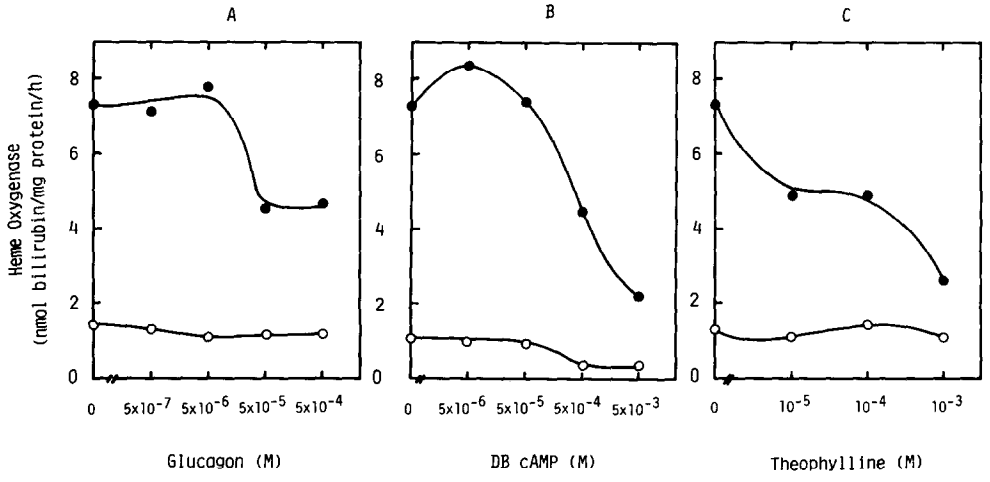

Fig. 5. Effects of glucagon (A) dibutyryl cAMP (B) and theophylline (C) on Co^{2+} induction of heme oxygenase in cultured and chick embryo liver cells. Cells were prepared and incubated as described in the text. Fresh medium without serum was added after incubation for 24 hr. The cells were then treated with $CoCl_2$ (50 μM) and other chemicals at concentrations indicated on the abscissa. After incubation for 24 hr, heme oxygenase activity was determined as described in the text. Data are expressed as the mean of duplicate determinations. Key: (○——○) control; and (●——●) Co^{2+} treated.

mediated by the trace metal, Co^{2+}, in cultured chick embryo liver cells. Since the studies were performed in hepatocyte cultures during incubation in a serum-free medium supplemented with the individual hormones, non-specific effects due to serum or extrahepatic factors which could modulate the heme oxygenase induction process were excluded. The hormones found to exert a significant regulatory action on basal heme oxygenase activity were insulin, hydrocortisone, triiodothyronine and dibutyryl cAMP. The induction of heme oxygenase in chick embryo liver cells was also greatly influenced by insulin, hydrocortisone, dibutyryl cAMP, glucagon, theophylline, epinephrine and norepinephrine in various ways.

Previous studies showed that adrenalectomy enhances basal as well as Co^{2+}-induced heme oxygenase in rat liver [14]. This suggested a role for hydrocortisone in regulating this enzyme *in vivo*. Administration of hydrocortisone to adrenalec-

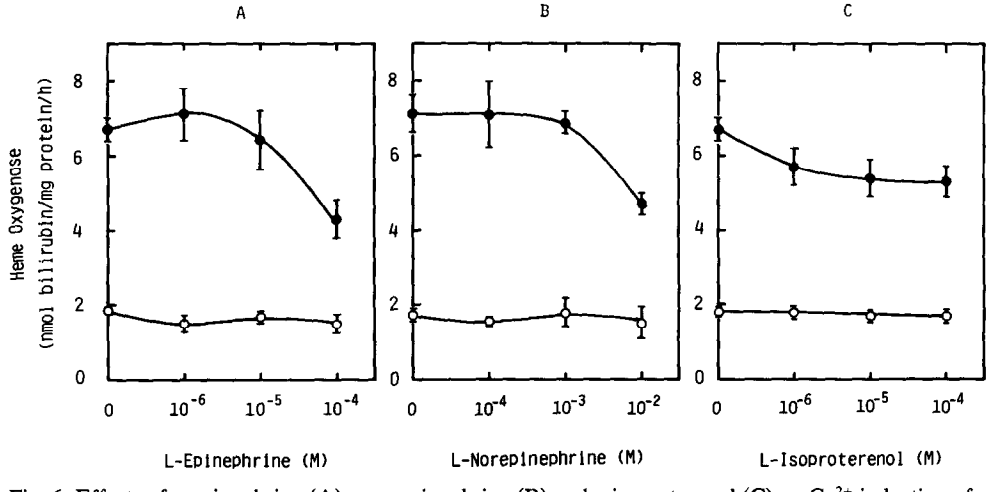

Fig. 6. Effects of L-epinephrine (A) L-norepinephrine (B) and L-isoproterenol (C) on Co^{2+} induction of heme oxygenase in cultured chick embryo liver cells. Cells were prepared and incubated as described in the text. After 24 hr of incubation, the medium was discarded and fresh medium without serum was added. The cells were treated with $CoCl_2$ (50 μM) and other chemicals at concentrations indicated on the abscissa. After 24 hr of incubation, heme oxygenase activity was determined as described in the text. Data are expressed as the means ± S.E. for three determinations. Key: (○——○) control; and (●——●) Co^{2+} treated.

tomized rats blocked the exaggerated induction response of heme oxygenase due to Co^{2+}. This suppressive effect of hydrocortisone on heme oxygenase was in contrast to the permissive action of the hormone on the induction of δ-aminolevulinate synthase in response to porphyrinogenic chemicals [29]. There are a number of reports describing the interrelationships of the secretory activities of the adrenal and thyroid glands [30–33]. It has been shown in particular that adrenalectomy causes an increase in plasma triiodothyronine levels and enhanced thyroid stimulating hormone responsiveness to thyrotropin releasing hormone [34]. Therefore, an increase in heme oxygenase activity after adrenalectomy could be attributable in part to enhanced triiodothyronine release or to decreased hydrocortisone levels. Cellular heme has been postulated to be involved in the repression of δ-aminolevulinate synthase, the rate-limiting enzyme in the heme biosynthetic pathway. Thus, the increased degradation of heme caused by induced heme oxygenase activity may be accompanied by alterations in δ-aminolevulinate synthase activity and cytochrome P-450 content in the liver [10, 11]. It was demonstrated earlier that insulin, hydrocortisone and triiodothyronine alone had no appreciable effects on the basal δ-aminolevulinate synthase level [17]. However, when insulin was added to liver cell cultures treated with allylisopropylacetamide, there was about a 20-fold increase in δ-aminolevulinate synthase [17]. The induction was much more pronounced when insulin and hydrocortisone or insulin, hydrocortisone and triiodothyrone were used in combination. The results demonstrated a permissive action of the hormones in the induction mechanism for δ-aminolevulinate synthase. The results of the present study, on the other hand, rule out a comparable permissive action of insulin, hydrocortisone or triidothyronine in relation to the induction of heme oxygenase by Co^{2+}. Rather, addition of either insulin or hydrocortisone to normal cultured cells caused a marked suppression of basal heme oxygenase activity. This finding suggests a possible dual role of insulin and hydrocortisone in the maintenance of cellular heme levels by increasing δ-aminolevulinate synthase induction and by suppressing heme oxygenase. The suppression of heme oxygenase activity by insulin in liver cells in culture contrasts with the *in vivo* effect of insulin administration in rats where insulin (1–12 I.U./100 g body wt) has been reported to cause an enhancement (2- to 7-fold) in heme oxygenase activity [12].

The results of the present study demonstrate that the addition of dibutyryl cAMP to cultured chick embryo liver cells inhibited Co^{2+} induction of heme oxygenase and also lowered the levels of heme oxygenase in untreated cultures (Fig. 5B). Similarly, this inhibitory effect on heme oxygenase induction was demonstrated by glucagon, theophylline and epinephrine, suggesting that a reciprocal relationship may exist between the induction of δ-aminolevulinate synthase and that of heme oxygenase activities in response to a cAMP-mediated process. Induction of heme oxygenase in macrophages caused by erythrophagocytosis is also suppressed by cAMP [35]. Intracellular cAMP concentrations may thus play a significant role in the maintenance of an intracellular heme pool, by regulating δ-aminolevulinate synthase and heme oxygenase activities in cultured liver cells.

These observations extend the spectrum of hormones now known to influence heme metabolism in the liver [36] and emphasize the importance of the endocrine milieu of this organ in determining its biochemical responses to environmental factors which lead to perturbations of hepatic heme and cytochrome P-450.

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