Receptor/Gene-Mediated Pharmacodynamic Effects of Methylprednisolone on Phosphoenolpyruvate Carboxykinase Regulation in Rat Liver


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ABSTRACT

Phosphoenolpyruvate carboxykinase (PEPCK) is the rate-limiting enzyme for gluconeogenesis. To investigate underlying mechanisms of corticosteroid (CS) action in regulating glucose, temporal patterns of hepatic PEPCK gene expression, enzyme activity, and cAMP content were examined in adrenalectomized rats receiving acute and chronic methylprednisolone (MPL) treatments. After single MPL intravenous doses, PEPCK mRNA showed a fast increase, reaching a maximum at around 0.75 h, which was followed by an immediate decline to below baseline after 4 h, an apparent acute tolerance/rebound phenomenon. However, PEPCK enzyme showed continuous hyperactivity for over 72 h. This may be the result of generation of cAMP, an important inducer of PEPCK activity, which peaked at around 6 h. During 7-day subcutaneous infusion of MPL, PEPCK mRNA showed profiles consistent with single-dose results, whereas PEPCK activity increased to a comparable maximum followed by a slow decline. However, the extent of cAMP induction was markedly higher during infusion, which could be attributed to amplification of cAMP synthesis and/or a stabilizing effect of MPL on cAMP degradation. A pharmacokinetic/pharmacodynamic model was developed based on receptor/gene mechanisms of CS action. It successfully described the dual effects of MPL on regulating PEPCK message and the post-transcriptional control by cAMP. Our results exemplify the importance of the extent and duration of steroid exposure in mediating pharmacological effects. The model provides quantitation of multiple controlling factors regulating PEPCK and presents insights into its function in glucose metabolism.

Corticosteroids are widely used for their anti-inflammatory and immunosuppressive effects. The major function of the endogenous hormones is to ensure an adequate glucose supply, especially under stress and starvation, by increasing gluconeogenesis (Baxter, 1976). Their metabolic roles become exaggerated upon chronic therapy, leading to numerous adverse effects such as osteoporosis, muscle wasting, and steroid-diabetes (Schimmer and Parker, 1996).

Both the therapeutic and adverse effects of CS are produced by their binding to glucocorticoid receptors (GRs) in target cells. Upon binding, they may cause rapid effects such as cortisol suppression and cell trafficking. The drug-receptor complex may further regulate gene expression in the nucleus and produce delayed alterations of various proteins (Jusko, 1994). Regulation by CS of many functional proteins, including enzymes, receptors, and cytokines has been extensively studied. However, limited information is available regarding their in vivo temporal patterns. Furthermore, little effort has been made to quantify and describe the intermediate processes of CS action. Mathematical description of the biological system and drug effects via mechanistic PK/PD models is crucial for quantitative understanding of the underlying mechanisms and reliable prediction of treatment outcome. Our laboratory has explored the time profiles of several proteins and their message expression after CS treatment in rats, including GR, tyrosine aminotransferase (TAT), and glutamine synthetase (Sun et al., 1998a, 1999; Ramakrishnan et al., 2002b). A series of PK/PD models describe the major rate-limiting steps of delayed steroid effects, including receptor binding, transcriptional down-regulation of GRs, mRNA induction, and enzyme induction.

ABBREVIATIONS: CS, corticosteroid; GR, glucocorticoid receptor; PK/PD, pharmacokinetic/pharmacodynamic; TAT, tyrosine aminotransferase; PEPCK, phosphoenolpyruvate carboxykinase; ADX, adrenalectomized; MPL, methylprednisolone; GRE, glucocorticoid-responsive element; TC, hypothetical transit biosignal; DR, cytosolic drug-receptor complex; DR(N), drug-receptor complex in nucleus.
Although TAT is a useful biomarker to study genomic effects of CS, it has the weakness of lacking direct clinical relevance. Hepatic TAT is the rate-limiting factor controlling tyrosine metabolism. Although this reaction generates a precursor for gluconeogenesis, it plays a minor role in overall glucose regulation.

Phosphoenolpyruvate carboxykinase catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, the rate-limiting step in gluconeogenesis. The CS increases gluconeogenic substrate availability and induces this key enzyme (Baxter, 1976). Given its central role in gluconeogenesis, it is not surprising that PEPCK is tightly regulated by various hormones, including CS, glucagon (via cAMP), and insulin.

In this study, dynamic changes in hepatic PEPCK mRNA expression and enzyme activity were examined using tissue samples from studies where rats received single injections or 7-day infusions of methylprednisolone. Cyclic AMP was also monitored as an important inducer for PEPCK. Additional animal studies were performed at selected times to validate experimental findings and the established model. Liver was selected because of its predominant role in gluconeogenesis and metabolic regulation. Rats were adrenalectomized to eliminate influences of endogenous corticosterone. Our results reveal that the regulation of PEPCK by CS was much more complex than TAT. The elevation of hepatic cAMP suggests possible secondary regulation of diverse genes/proteins after CS administration. The PK/PD model attempted to capture the major controlling mechanisms of PEPCK by CS and cAMP. Our model exemplifies the mathematical description of gene/protein regulation by multiple endogenous and exogenous substances and can be generalized to describe other drug actions.

Materials and Methods

Animals

Male adrenalectomized (ADX) Wistar rats with body weights of 271 ± 26 g (nine rats) or 367 ± 24 g (nine rats) were purchased from Harlan (Indianapolis, IN). Rats were adrenalectomized by the vendor 1 week before shipment. All animals were housed in a 12-h light/dark cycle, constant-temperature (22°C) environment with free access to rat chow and 0.9% NaCl drinking water. Animals were allowed to acclimatize to this environment for at least 1 week. Therefore, studies started at least 2 weeks after rat adrenalectomy, assuming steady-state of the ADX-induced dynamic changes had been achieved. One day before the study, nine rats (271 ± 26 g) underwent right external jugular vein cannulation under ketamine/xylazine anesthesia. Cannula patency was maintained with sterile 0.9% NaCl solution. This research adheres to the Principles of Laboratory Animal Care (NIH publication 85-23, revised 1985) and was approved by the University at Buffalo Institutional Animal Care and Use Committee.

Experimental

In the validation study, methylprednisolone sodium succinate (Solu-Medrol; Pharmacia and Upjohn Company, Kalamazoo, MI) was reconstituted with supplied diluents. Eighteen rats were divided into four groups: 1) Six cannulated rats received a single injection of 50 mg/kg MPL via the cannula over 30 s. Three rats were sacrificed by aortic exsanguination under ketamine/xylazine anesthesia at 6 h followed by the remaining three at 24 h. 2) Three cannulated rats were sacrificed without drug administration as a control group for the single-dose study. 3) Six rats received MPL 7-day infusion at a constant rate of 0.3 mg/kg/h via Alzet osmotic mini-pumps (model 2001, flow rate 1 μl/h; Alza, Palo Alto, CA) implanted subcutaneously under ketamine/xylazine anesthesia. For each rat, the concentration of the pump solution was prepared based on its predose body weight. Three rats were sacrificed at 24 h followed by the remaining three at the end of infusion (168 h). 4) Three rats were implanted with saline-filled pumps as a control group for the infusion study and sacrificed at various times throughout the 7-day period. For all animals, blood drained at sacrifice from the abdominal aortic artery was collected into a heparinized syringe and centrifuged immediately. Plasma samples were stored at −20°C for MPL measurements. Liver was rapidly excised, weighed, and flash frozen in liquid nitrogen for cAMP and PEPCK activity assays. Frozen liver tissues were ground into powder using a liquid nitrogen-chilled mortar and pestle.

Liver samples stored at −80°C were saved from previous studies in our laboratory. These included two single-dose studies where intravenous injection of 10 or 50 mg/kg MPL was administered to male ADX Wistar rats (225–250 g) via cannula (Sun et al., 1998a,b), and a 7-day infusion study where MPL was administered at constant rates of 0.1 or 0.3 mg/kg/h via subcutaneously implanted Alzet osmotic pumps to male ADX Wistar rats (300–350 g) (Ramakrishnan et al., 2002b). Two dose levels were included for each treatment paradigm to cover a wide range of drug concentrations and to assess dose-response relationships. Hepatic cAMP, PEPCK mRNA, and PEPCK activity were measured in these samples.

Drug Assay

Plasma MPL concentrations from the current study were determined by a normal-phase high-performance liquid chromatography method with a quantitation limit of 10 ng/ml as described previously (Haughey and Jusko, 1988).

cAMP Assay

A competitive enzyme-linked immunosorbent assay kit (Correlate-EIA Direct cAMP kit; Assay Designs, Inc., Ann Arbor, MI) was used for quantitative determination of hepatic cAMP. In brief, 0.5 g of frozen liver powder was homogenized in 5 ml of ice-cold 0.1 N HCl, which can inactivate phosphodiesterase and extract cAMP from cells (Brooker, 1982). The homogenates were first spun at 22,897 g for 30 min followed by a 105,156 g spin for 1 h at 4°C. Then, 100 μl of the final supernatant was incubated with polyclonal rabbit anti-cAMP antibody and alkaline phosphatase-conjugated cAMP for 2 h in a 96-well transparent plate coated with goat anti-rabbit IgG. The cAMP in the sample competed with the enzyme-linked cAMP for binding to the cAMP antibody. After washing, the enzyme reaction generated a yellow color, which was read at 405 nm with a microplate reader. The optical density was inversely proportional to cAMP content, and the sample concentrations were calculated based on standard curves. The assay has a sensitivity of 0.39 pmol/ml. Final results were converted into picomoles of cAMP per gram of liver.

PEPCK mRNA Assay

Quantitative Northern hybridization for measuring PEPCK mRNA was similar to the TAT mRNA assay established previously in our laboratory (DuBois et al., 1995). In brief, a cRNA pseudomessage standard, grg-1 cRNA, was added to the liver samples as an external standard before total RNA was extracted using a TRizol reagent (Invitrogen, Carlsbad, CA). Extracted total hepatic RNA, PEPCK cRNA, and grg-1 cRNA standards underwent electrophoresis on the same agarose formaldehyde gel and then were transferred to a nylon matrix (Duralon UV; Stratagene, La Jolla, CA) using a positive pressure transfer apparatus. Subsequent Northern hybridization was performed with 32P-labeled PEPCK and grg-1 cDNA probes. The hybridization signals were quantitated using a PhosphorImaging system (Amersham Biosciences Inc., Piscataway, NJ) and converted into moles of mRNA per gram of liver tissue.
PEPCK Activity Assay

Hepatic PEPCK activity was analyzed by modified Wimmer’s three-step luminometric method monitoring the stoichiometric transformation of oxaloacetate to phosphoenolpyruvate and then to ATP (Wimmer, 1988). Briefly, 0.5 g of liver powder stored at −80°C was homogenized in 4.5 ml of ice-cold 50 mM Hepes buffer (pH 7.5) and centrifuged at 105,180 g (4°C) for 60 min to obtain tissue cytosol. Then, 10 μl homogenate was added to a 96-well transparent plate in duplicate and incubated in a 52-μl reaction mix containing 50 mM potassium phosphate buffer, 10 mM MgSO4, 0.1 mM MnSO4, 0.1 mM EGTA, 1 mM mercaptoethanol, 0.05% bovine serum albumin, and 2.5 mM oxaloacetate. Oxaloacetate was metabolized to phosphoenolpyruvate by PEPCK in the sample. This reaction was initiated by adding 34.5 μl of 5 mM inosine-5'-triphosphate. The mixture was incubated at 25°C for 30 min. The reaction was terminated by adding 34.5 μl of 250 mM Na3PO4/K2HPO4 (pH 12) and subsequent heating to 70°C for 10 min. Next, the sample was brought to neutral pH by 34.5 μl of 0.25 N HCl and incubated with 34.5 μl of media containing 50 mM K2HPO4/KH2PO4 (pH 7), 2.5 mM KCl, 5 mM McCl2, 0.05% bovine serum albumin, 2 mM ADP, and 0.05 U of pyruvate kinase for 45 min at 25°C. The reaction of pyruvate kinase mediated formation of ATP from phosphoenolpyruvate and ADP and was terminated by adding 34.5 μl of 0.7 N NaOH. Then, 50 μl of reaction solution was transferred to a 96-well transparent plate in duplicate and incubated in a 52°C water bath for 30 min. ATP was determined via reaction with 50 μl of luciferase reagent using the ATP bioluminescence assay kit CLS II (Roche Diagnostics, Indianapolis, IN). The peak signal of emitted light was measured by microplate spectrophotometer SPECTRAMax GEMINI (Amersham Biosciences Inc.), and data were analyzed by SOFTmax PRO version 2.6.1 (Amersham Biosciences Inc.). Blank reactions were performed using liver samples incubated without oxaloacetate and inosine-5'-triphosphate. All sample measurements were corrected by their corresponding tissue blanks for adjustment of the ATP contents in liver. The reaction was controlled by adding 10 μl of known phosphoenolpyruvate as well as ATP to the first step of the reaction mixture instead of the tissue sample. Assay results were expressed as micromoles of ATP formed per minute per gram of liver.

Pharmacokinetic/Pharmacodynamic Model

Pharmacokinetics. The PK equations and parameter values were obtained from the original studies published previously by our laboratory (Table 1). The biexponential kinetics of MPL in plasma (C MPL ) after 50 mg/kg i.v. injection was described by Sun et al. (1998a).

\[
C_{\text{MPL}} = C_1 \cdot e^{-x_1 \cdot t} + C_2 \cdot e^{-x_2 \cdot t} \tag{1}
\]

where \( C_1 \) and \( A_1 \) are the coefficients for the intercepts and slopes.

A two-compartment model with rapid i.v. input (10 mg/kg injection study) or zero-order input (infusion study) into the central compartment was used to describe the kinetics (Sun et al., 1998b; Ramakrishnan et al., 2002b):

\[
\frac{dA_t}{dt} = k_{21} \cdot A_t - k_{12} \cdot A_t - \left( \frac{CL}{V_p} \right) \cdot A_p - k_e [\text{infusion study}] \tag{2}
\]

\[
\frac{dA_t}{dt} = k_{12} \cdot A_p - k_{31} \cdot A_t \tag{3}
\]

\[
C_{\text{MPL}} = A_p / V_p \tag{4}
\]

where \( A_p \) and \( A_t \) represent the amount of drug in the plasma and tissue compartments. Other parameters include the drug clearance \( CL \), the central volume of distribution \( V_p \), and the distribution rate constants \( k_{12} \) and \( k_{21} \). For the infusion study, \( k_e \) is the zero-order input rate constant.

Plasma drug concentrations over time were fixed to drive the dynamics in the following data analysis.

Mechanistic Basis for Pharmacodynamics. The cellular processes for CS pharmacodynamics are depicted in Fig. 1. Free CSs in blood are moderately lipophilic and can diffuse into liver cells. These steroids quickly bind to cytosolic GRs and subsequently activate the receptors. The receptors undergo conformational change by dissociating from heat-shock proteins 90, 70, and 56, followed by phosphorylation (Hogger and Rohdewald, 1998). The activated steroid-receptor complexes rapidly translocate into the nucleus where they bind as dimers to glucocorticoid responsive elements (GREs) in the target DNA. After the transcriptional control of target genes, the steroid-receptor complexes in the nucleus may dissociate from GREs, and return to the cytosol. Part of the receptors may be degraded during the process, whereas the rest may be reassembled with heat-shock proteins and recycled.

Upon binding to GREs in nucleus, the activated steroid-receptor complexes may interact with RNA polymerase complexes or regulatory proteins located in the DNA control regions and affect the transcription of several RNAs. The RNA transcripts undergo 5’ capping, splicing, and 3’ polyadenylation to produce mRNAs. The mRNAs are exported to the cytoplasm where they can be degraded or translated into proteins. Thus, transcriptional regulation by CS may yield altered mRNA and protein levels. CSs are known to cause homologous down-regulation of their own receptors via decreased transcription, which subsequently results in decreased mRNA levels and free GR densities in the cytosol (Oakley and Cidlowski, 1993). The CS can also induce adenyl cyclase and suppress phosphodiesterase based on similar transcriptional control (Baus et al., 2001). Adenyl cyclase catalyzes the conversion of ATP to cAMP, which is degraded by phosphodiesterase (Buchter and Butcher, 1968). Alterations of these two enzymes by CS may result in increased cAMP levels in target cells. It is recognized that CS can enhance PEPCK gene transcription (Granner et al., 1986). The increased degradation rate of this message in liver after CS treatment has also been reported to occur via an unknown mechanism (represented by hypothetical biosignal TC in Fig. 1) (Hoppen et al., 1986). The translation of PEPCK mRNA to protein is known to be up-regulated by cAMP (Gunn et al., 1975b). In addition, cAMP may reduce the activity of PEPCK enzyme (Gunn et al., 1997). These actions result in the multifaceted regulation of PEPCK via multiple processes.

Receptor Dynamics. Based on the cellular mechanisms of steroid action, a PD model as depicted in Fig. 2 was used to describe receptor dynamics in rat liver after MPL treatments. This was partially adapted from our most current model for CS pharmacodynamics using receptor

### Table 1

<table>
<thead>
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<th>Parameter</th>
<th>Value</th>
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<td><strong>Pharmacokinetics</strong></td>
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<td>C2 (ng/ml)</td>
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<td>λ2 (h⁻¹)</td>
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<tr>
<td>kₚₚ (h⁻¹)</td>
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<tr>
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<tr>
<td>Rₚ (fmol/mg protein)</td>
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<tr>
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<tr>
<td>Sₚ (l/(mg/mg protein)⁻¹)</td>
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<table>
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<td>a 10 mg/kg i.v. injection; b 50 mg/kg i.v. injection; c infusion; d validation study (injection); e validation study (infusion).</td>
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Fig. 1. Schematic representation of diverse molecular and cellular mechanisms of CS action on cAMP and PEPCK in hepatocytes. AC, adenylyl cyclase; PDE, phosphodiesterase.

Fig. 2. Receptor/gene-mediated model of CS pharmacodynamics in regulating PEPCK. Symbols and differential equations for the model are defined in eqs. 1 to 19. The dotted lines and rectangles indicate stimulation (open bar) and inhibition (solid bar) of the various processes via indirect mechanisms. Names with gray shading represent PD markers corrected for liver hypertrophy during drug infusion.
mRNA and density data (Ramakrishnan et al., 2002a). The differential equations for receptor dynamics include the following:

\[
\frac{\text{d} \text{mRNA}_R}{\text{d}t} = k_{\text{p}m}^{\text{R}} \cdot \left( 1 - \frac{\text{DR}(N)}{\text{IC}_{50}^{\text{mRNA}} + \text{DR}(N)} \right) - k_{\text{d}m}^{\text{R}} \cdot \text{mRNA}_R \quad (5)
\]

\[
\frac{\text{d} \text{R}}{\text{d}t} = k_{\text{p}R} \cdot \text{mRNA}_R + R_f \cdot k_{\text{a}R} - k_{\text{d}R} \cdot \text{R} \quad (6)
\]

\[
\frac{\text{d} \text{DR}(N)}{\text{d}t} = k_{\text{a}R} \cdot \text{DR} - k_{\text{d}R} \cdot \text{DR}(N) \quad (7)
\]

where symbols represent the plasma molar concentration of MPL (D), the receptor mRNA (mRNA_R), the free cytosolic GR density (R), cytosolic drug-receptor complex (DR), and receptor-complex in nucleus (DR(N)). The rate constants in the equations include zero-order rate of GR mRNA synthesis (\(k_{\text{p}m}^{\text{R}}\)), the first-order rates of GR mRNA degradation (\(k_{\text{d}m}^{\text{R}}\)), receptor synthesis (\(k_{\text{p}R}\)) and degradation (\(k_{\text{d}R}\)), translocation of the drug-receptor complex into the nucleus (\(k_{\text{a}R}\)), the overall turnover of DR(N) return receptors to cytosol (\(k_{\text{a}R}\)), as well as the second-order rate constant of drug-receptor association (\(k_{\text{a}R}\)). In addition, IC_{50}^{\text{mRNA}} is the concentration of DR(N) causing 50% inhibition of the synthesis rate of receptor mRNA, and \(R_f\) is the fraction of free receptor being recycled.

Equations 5 and 6 yield the following baseline equations:

\[
k_{\text{d}m}^{\text{R}} = \frac{k_{\text{d}m}^{\text{R}}}{\text{mRNA}_R} \quad (9)
\]

\[
k_{\text{p}R} = \left( \frac{R_L^0}{\text{mRNA}_R} \right) \cdot k_{\text{p}R} 
\quad (10)
\]

where mRNA_R and R_L^0 are the baseline values of receptor mRNA and free cytosolic GR density. These baseline values were fixed as the mean values obtained in tissues from the control animals in previous reports (Sun et al., 1998b; Ramakrishnan et al., 2002b). Parameters reported in the fifth generation model (Ramakrishnan et al., 2002a) were used to simulate receptor dynamics and produce the driving force in the present study (Table 1).

**Liver Weight.** As depicted in Fig. 2, the changes in liver weight during MPL infusion was described by a model established previously for these data (Ramakrishnan et al., 2002b):

\[
\frac{\text{d} \text{Lwt}}{\text{d}t} = k_L^\text{L} \cdot (1 + S_L^0 \cdot \text{DR}(N)) - k_{\text{d}L} \cdot \text{Lwt} 
\quad (11)
\]

where Lwt is liver weight ratio representing the ratio of the measured organ weight to the estimated predose organ weight (Ramakrishnan et al., 2002b). Liver weight was produced at a zero-order rate \(k_L^\text{L}\) and diminished via a first-order rate constant \(k_{\text{d}L}\). The drug-receptor complex in the nucleus DR(N) was responsible for the liver hypertrophy by stimulating weight production with a linear efficiency factor \(S_L^0\).

At time 0, the system baseline was described by the following:

\[
k_L^\text{L} = k_L^\text{L} \cdot \text{Lwt}_0 
\quad (12)
\]

Baseline liver weight ratio Lwt_0 was fixed as 1. Parameters reported in our previous study (Ramakrishnan et al., 2002b) were used to simulate liver weight change (Table 1).

**Induction of cAMP.** Inhibition of cAMP degradation rate (\(k_{\text{d}C}^\text{AMP}\)) by the drug-receptor complex in the nucleus DR(N) was assumed to be the major mechanism of cAMP increase as follows (Fig. 2):

\[
\frac{\text{d} \text{cAMP}}{\text{d}t} = k_L^\text{CAMP} \cdot (1 - \frac{\text{DR}(N)}{\text{IC}_{50}^{\text{CAMP}} + \text{DR}(N)}) \cdot \text{cAMP} 
\quad (13)
\]

where cAMP is the hepatic cAMP concentration in picomoles per gram of liver. Endogenous cAMP is produced at a constant rate \(k_L^\text{CAMP}\). The IC_{50}^{\text{CAMP}} represents the concentration of DR(N) producing 50% inhibition of cAMP degradation.

The cAMP level was assumed to be at steady-state at time 0 (control animals), yielding the following baseline equation:

\[
k_L^\text{CAMP} = k_L^\text{CAMP} \cdot \text{cAMP}_0 
\quad (14)
\]

The baseline value of cAMP (\(\text{cAMP}_0\)) was fixed as the mean of the control animals from each study.

The proposed model (eq. 13) was applied to the cAMP measurements using stored liver samples. The PD model containing DR(N) stimulation of cAMP production was also tested. Drug action was examined using different mathematical functions, including linear and sigmoidal relationships with or without a Hill factor. Once the optimal model was established and parameter estimates were obtained, they were fixed in the following data analysis.

**Induction of PEPCK mRNA.** The CS may affect PEPCK mRNA by dual mechanisms. The following equations were used to describe simultaneous actions of CS on PEPCK mRNA synthesis and degradation (Fig. 2):

\[
\frac{\text{d} \text{mRNAPEPCK}}{\text{d}t} = k_{\text{p}m}^{\text{PEPCK}} \cdot (1 + S_{\text{m}0}^\text{PEPCK} \cdot \text{DR}(N)) - k_{\text{d}m}^{\text{PEPCK}} \cdot \text{mRNAPEPCK} 
\quad (15)
\]

where TC is the concentration of the presumed biosignal responsible for the CS stimulation of PEPCK mRNA degradation. A linear transduction model (Sun and Jusko, 1998) was used to describe this biosignal, which was generated from DR(N) at the first-order rate \(k_L\). The mRNAPEPCK is the PEPCK message level in liver expressed as femtomoles per gram of liver. The stimulation of PEPCK mRNA synthesis rate \(k_{\text{p}m}^{\text{PEPCK}}\) is dependent on DR(N) concentration with a linear efficiency factor \(S_{\text{m}0}^\text{PEPCK}\), and the stimulation of PEPCK mRNA degradation rate \(k_{\text{d}m}^{\text{PEPCK}}\) is dependent on the transient TC with a linear efficiency factor \(S_{\text{m}0}^\text{PEPCK}\).

The mRNAPEPCK message level was assumed to be at steady-state at time 0 (control animals), yielding the following baseline equation:

\[
k_{\text{p}m}^{\text{PEPCK}} = k_{\text{p}m}^{\text{PEPCK}} \cdot \text{mRNA}_{0}^\text{PEPCK} 
\quad (17)
\]

Baseline message level mRNA_{0}^\text{PEPCK} was fixed as the mean from control animals in each study.

Equations 15 and 16 were used to fit the PEPCK mRNA measurements from stored liver samples. The obtained parameters were fixed to produce PEPCK mRNA dynamics in the following data analysis.

**Induction of PEPCK Activity.** The PEPCK enzyme was translated from its mRNA at the first-order rate \(k_L^\text{P}\) with amplification factor \(\gamma\) and degraded at the first-order rate constant \(k_{\text{d}P}\). The \(\gamma\) indicates that multiple copies of protein could be synthesized from a single mRNA transcript. The stimulation of this translation process by cAMP was assumed to be the major mechanism of cAMP action on PEPCK (Fig. 2). The equations for PEPCK dynamics are as follows:

\[
\frac{\text{d} \text{PEPCK}}{\text{d}t} = k_L^\text{P} \cdot \text{S}^\text{P}(t) \cdot (\text{mRNAPEPCK}) - k_{\text{d}P} \cdot \text{PEPCK} 
\quad (18a)
\]

\[
\text{S}^\text{P}(t) = 1 + S_{\text{m}0}^\text{PEPCK} \cdot (\text{cAMP} - \text{cAMP}_0) 
\quad (18b)
\]

The hepatic PEPCK activity is expressed as micromoles of ATP per minute per gram of liver. The \(S_{\text{m}0}^\text{PEPCK}\) and IC_{50}^{\text{CAMP}} represent the maximum possible stimulation of \(k_L^\text{P}\) and the elevated cAMP re-
quired for half-maximal stimulation. The change of cAMP from its
dynamic model. This effect was corrected by the following equation.

\[ k_P = k_P^0 \cdot \text{PEPCK}^0 \] (mRNA\text{PEPCK})^0 \quad (19) \]

The baseline value of PEPCK (PEPCK^0) was fixed as the mean of the
correction applied to the PEPCK activity measurements using stored liver samples. A PD model containing
inhibition of PEPCK degradation by cAMP was also tested. Drug action was examined by different mathematical functions, including linear and sigmoidal relationships with or without the Hill factor.

**Data Analysis.** Data in this study were generated from a so-called “giant rat” study in our laboratory. Animals were sacrificed to obtain serial blood and tissue samples. Each point represents the measurement from one individual rat, and data from all these different rats were analyzed together to obtain a time profile as though it came from one giant rat. A naive pooled data analysis approach was therefore used for all model fittings using ADAPT II software (D’Argenio and Schumitzky, 1997). The maximum likelihood method was used with variance model specified as \( V(\sigma^2, \text{Lwt}, \text{R}) = \sigma^2 \cdot \text{Y}(\theta, \text{Lwt})^2 \), where \( V(\sigma^2, \theta, \text{Lwt}) \) is the variance for the 4th point, \( \text{Y}(\theta, \text{Lwt}) \) is the 4th predicted value from dynamic model, \( \sigma \) represents the estimated structural parameters, and \( \sigma_r, \sigma_d \) are the variance parameters that were estimated.

Liver hypertrophy during long-term dosing would produce a dilution effect for hepatic PD markers that were expressed on a per organ weight basis. This effect was corrected by the following equation.

\[ R_{\text{output}} = R / \text{Lwt} \quad (20) \]

where \( R \) represents the cAMP, PEPCK mRNA, and PEPCK levels described by eqs. 13, 16, and 18 during MPL infusion. The Lwt is the liver weight ratio of corresponding treatments as defined in eq. 11. The \( R_{\text{output}} \) is the corrected value that was used as the observed data for the infusion study.

The measurements using stored liver samples from previously performed studies (two acute dosing studies and one infusion study) were used to test and compare the proposed models. Models were selected based on visual inspection of curve fitting, estimator criteria, Schwartz criterion, and confidence of parameter estimations. The parameter estimates from the final established PD model were used to perform simulations for the current animal study to validate the model. The baselines for these simulations were obtained from the mean data of present control animals, as listed in Table 2.

**Results**

Our PD model was developed based on analyzing stored liver samples from previously performed animal studies. The integrity of message level was demonstrated by the intact RNA bands in Northern hybridization. We found no significant change in cAMP level and PEPCK activity when liver samples were stored at \(-80^\circ\text{C} \) for up to 6 months (data not shown). McGraw et al. (1986) reported the PEPCK activity in liver was not significantly correlated with the length of storage at \(-70^\circ\text{C} \) for up to 21 days. Our assay results were further validated in the newly performed animal study in which PD markers were measured at selected time points.

**Pharmacokinetics.** Simulations of MPL pharmacokinetic profiles are shown in Fig. 3. The MPL plasma concentration after a single dose exhibited a biexponential decline with a terminal half-life of about 30 min. During infusion, MPL concentrations were maintained at steady-state, which was achieved soon after drug administration. Plasma MPL concentrations from the validation study correlated well with the model-predicted PK behavior.

**Receptor Dynamics.** Detailed descriptions of receptor dynamics in liver can be obtained from our previous publications (Sun et al., 1998a,b; Ramakrishnan et al., 2002b). In brief, receptor mRNA was down-regulated to a minimum around 10 h and returned close to baseline by 48 h after acute dosing. The free cytosolic receptor density fell immediately to zero and returned to baseline by 72 h in two phases, a fast initial rise for 8 h and a later slow return. During infusion, both receptor mRNA and receptor density fell to new steady states within a day.

**Liver Weight.** Simulation of the increases in liver weight during MPL infusion is shown in Fig. 4. Liver hypertrophy from the validation study was somewhat overpredicted by the reported parameters. This may be simply due to interanimal variability.

**TABLE 2**

Dynamic parameters for effects of methylprednisolone on PEPCK regulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP Dynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k_i^c ) (h(^{-1}))</td>
<td>0.27</td>
<td>17</td>
</tr>
<tr>
<td>( IC_{50}^c ) (fmol/mg protein)</td>
<td>433.4±h/17.81±e</td>
<td>29±%9/g</td>
</tr>
<tr>
<td>( k_{\text{off}}^c ) (pmol/g liver)</td>
<td>654.4±856.1±806.1±562.7±740.5±</td>
<td>17</td>
</tr>
<tr>
<td>PEPCK mRNA Dynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k_i ) (h(^{-1}))</td>
<td>0.75</td>
<td>13</td>
</tr>
<tr>
<td>( k_{\text{off}}^m ) (h(^{-1}))</td>
<td>0.017</td>
<td>38</td>
</tr>
<tr>
<td>( S_{\text{th}}^m ) (fmol/mg protein)(^{-1})</td>
<td>2.11</td>
<td>52</td>
</tr>
<tr>
<td>( S_{\text{th}}^m ) (fmol/mg protein)(^{-1})</td>
<td>2.90</td>
<td>51</td>
</tr>
<tr>
<td>PEPCK mRNA(^0) (fmol/g liver)</td>
<td>383.4±209.0±429.8±</td>
<td>Fixed</td>
</tr>
<tr>
<td>( k_{\text{inf}}^m ) (fmol/g liver/h)</td>
<td>6.46±3.52±7.24±</td>
<td>38</td>
</tr>
<tr>
<td>PEPCK Dynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k_i^p ) (h(^{-1}))</td>
<td>0.23</td>
<td>12</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>0.004</td>
<td>-9</td>
</tr>
<tr>
<td>( S^p )</td>
<td>1.17</td>
<td>10</td>
</tr>
<tr>
<td>( S_{\text{th}}^p ) (fmol/g liver)</td>
<td>0.0055</td>
<td>-9</td>
</tr>
<tr>
<td>PEPCK(^0) (\mu mol ATP/min/g liver)</td>
<td>1.40±h/1.83±1.98±/2.07±</td>
<td>Fixed</td>
</tr>
<tr>
<td>( k_{\text{inf}}^p ) (\mu mol ATP/min/g liver/h/fmol/g liver)</td>
<td>0.30±/0.38±</td>
<td>12</td>
</tr>
</tbody>
</table>

*10 mg/kg i.v. injection; *50 mg/kg i.v. injection; *infusion; *validation study (injection); *validation study (infusion); *secondary parameter; *not available.
Induction of cAMP. The speed of tissue fixation and cAMP extraction are critical factors in cAMP measurements. A clamping technique using two metal blocks and liquid nitrogen has been applied to measure cAMP in brain and heart (Robison et al., 1971a). Unlike brain, which contains high-affinity phosphodiesterase ($K_m = 3–8 \mu$M) and yields a cAMP half-life of less than a minute, liver contains low-affinity phosphodiesterase ($K_m = 50–200 \mu$M) and yields a longer cAMP half-life of 4 to 6 min (Robison et al., 1971a). Instead of clamping, we rapidly excised liver, which was flash frozen in liquid nitrogen. There was no change in cAMP level even if liver was kept at room temperature for up to 2 min (data not shown). Baseline cAMP was comparable with reported cAMP concentrations of 100 to 500 pmol/g tissue (Robison et al., 1971b).

Induction of cAMP by MPL administration is shown in Fig. 5. The cAMP concentration was temporarily increased from 654 ± 119 to 883 ± 161 pmol/g liver after the 10 mg/kg MPL i.v. dose, and from 656 ± 47 to 951 ± 113 pmol/g liver after 50 mg/kg MPL. The hepatic cAMP level peaked at around 6 h and returned to baseline after 24 h. During MPL infusion, cAMP level rose fast in the first 24 h from 806 ± 278 to 2671 ± 263 pmol/g liver with the low dose, and to 2716 ± 623 pmol/g liver with the high dose. The extent of cAMP induction during infusion was markedly higher than after the single dose. After 1 day, hepatic cAMP showed a slow decrease in spite of continuous drug administration, indicating development of some drug tolerance.

The proposed model (eq. 13) nicely captured the cAMP induction by MPL and Table 2 lists the parameter estimates. This model represented the final selection after comparing submodels based on other mechanisms. Different $IC_{50}^C$ values for acute and chronic dosing were necessary to fit cAMP profiles. This difference could be attributed to an increase in stabilization of cAMP by MPL after continuous exposure. The $IC_{50}^C$ estimate of 433 fmol/mg protein indicates that the effective drug-receptor complex in nucleus DR(N) did not achieve the concentration necessary for half-maximal inhibition of cAMP degradation after MPL single-dose (partly depicted in Fig. 7). This relatively large value of $IC_{50}^C$ explained the dose-dependent cAMP induction (Fig. 5, left). However, with continuous exposure, the DR(N) was maintained above the lowered $IC_{50}^C$ (17.8 fmol/mg protein) during the entire infusion (data not shown). This small value of $IC_{50}^C$ implies the saturation of cAMP induction by the MPL concentration during infusion and explains the relative lack of dose-response differences (Fig. 5, right). The tolerance phenomenon during long-term administration could be partly explained by the reduced driving force [DR(N)] because of down-regulation of glucocorticoid receptor mRNA and unavailability of free receptor. In addition, the dilution effect of liver hypertrophy also contributed to the slow decrease of the observed cAMP levels during infusion.

Induction of PEPCK mRNA. The regulation of PEPCK gene expression by MPL administration is shown in Fig. 6. The levels of PEPCK mRNA showed a fast increase by 0.75 h from 383 ± 247 to 759 ± 253 fmol/g liver after the 10 mg/kg MPL i.v. dose, and from 290 ± 178 to 794 ± 112 fmol/g liver after 50 mg/kg MPL. This peak at 0.75 h was followed by an immediate decline to lower than baseline after 4 h, an acute tolerance/rebound phenomenon. During MPL infusion, PEPCK message levels were maintained below baseline. The
The alterations of PEPCK mRNA were well described by the proposed model (eqs. 15 and 16) with parameters listed in Table 2. The transcriptional control of MPL via DR(N) produced the initial induction. The hypothetical biosignal TC produced by linear transduction was responsible for the abrupt decrease in PEPCK mRNA levels, which explained the acute tolerance phenomenon [earlier PEPCK mRNA peak than DR(N) peak]. Simulations illustrating the contributions of DR(N) and TC on regulating gene expression are shown in Fig. 7. The time delay between the two driving forces that was introduced by the transduction process \( k_1 \) ensured the message enhancement by DR(N). The efficiency of MPL action on PEPCK mRNA degradation via TC \( S^{\text{TC}}_{\text{pm}} = 2.90 \text{ (fmol/mg protein)}^{-1} \) is higher than its action on mRNA synthesis via DR(N) \( S^{\text{DR}}_{\text{pm}} = 2.11 \text{ (fmol/mg protein)}^{-1} \). This efficiency difference yielded the later message down-regulation. The less gene induction after high dose of MPL i.v. injection (50 mg/kg) than low dose (10 mg/kg) was partly due to the lower baseline PEPCK mRNA level (290 versus 383 fmol/g liver), which would mathematically yield a lower PEPCK mRNA synthesis rate \( k^\text{pm} \) (eq. 17). In addition, the high dose of MPL produced higher TC concentrations (Fig. 7), which would in turn stimulate mRNA degradation and yield lower PEPCK mRNA level than low dose MPL.

**Induction of PEPCK Activity.** In spite of the transient induction of its mRNA, the PEPCK enzyme showed continuous hyperactivity after i.v. injection and during infusion (Fig. 8). The PEPCK activity was increased from 1.40 ± 0.47 to 2.96 ± 1.00 and 3.62 ± 1.14 μmol ATP/min/g liver at the end of study (72 h) after 10 and 50 mg/kg MPL single injections.
During MPL infusion, PEPCK enzyme activity rose in the first 10 h from 1.83 ± 1.82 to 4.79 ± 2.58 μmol ATP/min/g liver with the low dose, and to 6.19 ± 2.20 μmol ATP/min/g liver with the high dose. Hepatic PEPCK activity of both doses then exhibited a very slow decrease despite the continuous drug administration, indicating that some tolerance developed with chronic dosing.

The sustained elevation of PEPCK activity was caused by the MPL-induced cAMP level, an important inducer of this enzyme. The proposed model (eq. 18) well captured the PEPCK induction and Table 2 lists the fitted parameters. This model represents the final selection after comparing submodels based on other mechanisms. The estimated PEPCK degradation rate ($k_p^{\text{PEPCK}}$) of 0.22 h$^{-1}$ (half-life = 3 h) agrees well with an in vivo measurement of 0.1 h$^{-1}$ in rats (Gunn et al., 1975a). The small $\gamma$ value of 0.004 implies a very low translational efficiency of PEPCK mRNA. The low SC$^{\text{PEPCK}}_{50}$ of 0.0055 fmol/g liver suggests that even a small change in cAMP level will be sufficient to produce the maximal stimulation effect. This small value of SC$^{\text{PEPCK}}_{50}$ implies the saturation of PEPCK elevation by intracellular cAMP and explains the relative lack of dose-response differences. The low precision associated with some parameter estimates indicates that the model was somewhat over-parameterized partly due to data variability.

**Model Validation.** The results from the validation study are depicted in Fig. 9. The observed hepatic cAMP level and PEPCK activity at selected time points agreed well with the measurements using stored liver samples. This confirmed the stability of the samples. Simulations using the established model and parameter values reasonably captured the cAMP and PEPCK profiles in this animal study.

**Discussion**

Proteins can be regulated at multiple sites from DNA to protein: transcription, RNA processing, mRNA transport and degradation, translation, and protein degradation and activation. Upon binding to GRs, CS can regulate specific proteins via these mechanisms. In this study, hepatic PEPCK was selected as the PD marker because of its essential role in gluconeogenesis. The PEPCK gene expression was regulated by CS via dual mechanisms. Its transcriptional induction was very short lived because of the effect on decreasing mRNA stability. Interestingly, the sustained enzyme hyperactivity showed a discrepancy with its message behavior, suggesting additional post-transcriptional control. We found that hepatic cAMP levels increased with CS treatment. Due to the regulation of numerous genes and proteins by cAMP, our results suggested that in vivo drug effects such as the PEPCK induction may be secondary to the CS-induced cAMP.

The CS can increase cAMP accumulation in various cell types in vitro (Hege Thoresen et al., 1989; Yingling et al., 1994; Baus et al., 2001). Our results confirmed this phenomenon in vivo and showed its time pattern. The cAMP elevation by dexamethasone was abolished by the steroid antagonist RU486 or protein synthesis inhibitor cycloheximide (Baus et al., 2001), indicating that it required GR binding and was protein synthesis-dependent. Several mechanisms have been proposed concerning CS effect on cAMP, including increased formation, decreased degradation, as well as regulation of Gs protein. Baus et al. (2001) reported that dexamethasone-induced cAMP correlated with both an increase in adenylyl cyclase activity (cAMP-producing enzyme) and a decrease in phosphodiesterase activity (cAMP-degradation enzyme). However, Christoffersen et al. (1984) found dexamethasone decreased phosphodiesterase activity, with no significant effect on adenylyl cyclase. Phosphodiesterase mRNA was suppressed by dexamethasone (Yingling et al., 1994), suggesting that CS might modulate phosphodiesterase gene expression via DR(N). Both mechanisms of increased synthesis and decreased degradation were tested, and the model assuming inhibition of cAMP degradation better described our data. The markedly higher induction of cAMP during infusion and the different IC$^{\text{50}}_{50}$ estimates suggested that extra complexities were introduced during chronic treatment. Cyclic AMP is affected not only by CS but also by other factors. The extensive steroid effects may lead to changes of endogenous substances during chronic therapy, such as decreased insulin sensitivity (phosphodiesterase inducer).

As the rate-limiting enzyme for gluconeogenesis, PEPCK has been studied for decades. The CS and cAMP are two major inducers of PEPCK. It was generally hypothesized that CS promotes an increase in PEPCK mRNA, whereas cAMP enhances the translation of preexisting message. This served as the assumption for our PEPCK model. This hypothesis was supported by using specific inhibitors to differentiate CS and cAMP effects. In hepatocytes, perfused liver, and in vivo, PEPCK induction by CS was blocked by transcription inhibi-
itors actinomycin D and cordycepin (Krone et al., 1975; Sharma and Patnaik, 1984). The PEPCK promoter region contains the GRE sequence (O’Brien et al., 1995). These studies indicate that CS act at the transcriptional level. It was also reported that rat adrenalectomy resulted in a considerable prolongation of PEPCK mRNA half-life, and hydrocortisone increased PEPCK mRNA degradation rate in vivo (Hoppner et al., 1986). The enhancement of both mRNA synthesis and degradation enables the liver to respond rapidly to metabolic alterations. This dual action of CS was captured in our model by stimulation of synthesis via DR(N) and stimulation of degradation via DR(N)-produced-biosignal TC. The transduction process accounted for the delayed occurrence of down-regulation and captured the acute tolerance/rebound phenomenon after single doses. The continuous message suppression during infusion further confirmed this TC-mediated down-regulation.

Cyclic AMP, on the other hand, can increase PEPCK activity even with the addition of actinomycin D or cordycepin (Krone et al., 1975). Removal of cAMP during the early phase of PEPCK increase prompted an immediate cessation of the induction, implying that cAMP might act at post-transcriptional level instead of by transcriptional stimulation, which would produce continuous PEPCK hyperactivity after cAMP removal (Wicks, 1971). The PEPCK induction by cAMP was rapid with little lag time, in contrast to the slower CS response (Wicks, 1974). Gunn et al. (1975b) suggested that cAMP might take effect between the action sites for cordycepin [poly(A) tail addition to mRNA] and cycloheximide (peptide chain elongation). One suitable step is the initiation of

**Fig. 8.** Hepatic PEPCK activity versus time profiles upon MPL administration of 10 (●) and 50 (○) mg/kg injection, or 0.1 (▲) and 0.3 (△) mg/kg/h infusions for 7 days. Lines are results of the simultaneous fittings with eqs. 18 to 20. Solid lines represent injection groups. Broken lines represent MPL infusion groups. Thin lines represent low-dose groups and thick lines represent high-dose groups. The PD parameters are listed in Table 2.

**Fig. 9.** Hepatic cAMP (left) and PEPCK activity (right) profiles from the validation study where rats received MPL 50 mg/kg injection (○) or 0.3 mg/kg/h 7-day infusion (△). Symbols are the mean data and errors are the standard deviations.
protein synthesis. The authors also reported that cAMP reduced enzyme stability (Gunn et al., 1976). The increased PEPCK activity by cAMP was completely prevented by cycloheximide (Barnett and Wicks, 1971), and this induction was not associated with alterations in enzyme properties (Wicks et al., 1972). These suggest that cAMP does not activate existing proteins. Both mechanisms of increased translation and decreased protein degradation were tested, and the model assuming translational stimulation by cAMP better described the data. Our parameter estimates implied that this translation had very low efficiency and was very sensitive to cAMP modulation. The increased cAMP was thus deemed responsible for the sustained PEPCK hyperactivity after MPL treatment.

Our model well described the induction of cAMP, PEPCK message, and activity by CS and was validated in a follow-up experiment. The model was constructed using a piecewise fitting technique in which estimated dynamics were fixed in the subsequent data analysis. This technique was necessary for this very complex model. To our knowledge, this is the first PD model incorporating the multihormonal regulation of multiple processes of a specific protein based on known mechanisms. However, our model assumed DR(N) was the only mediator for cAMP induction, and the tolerance phenomenon during infusion was due to GR saturation and down-regulation, plus the dilution effect of liver weight change. As an essential second messenger, cAMP may be altered by many other factors such as activation of any G protein receptors. Administered CS may affect a number of enzymes/hormones and thus cause secondary changes of cAMP level. Such additional mechanisms may contribute to both the increased cAMP accumulation and the tolerance development. Our model was limited by assuming the high MPL concentrations dominate other factors in regulating cAMP and overlook extra complexities. The result IC₅₀ may be composite parameter representing a combination of primary and secondary actions of MPL. In addition, consistent with its key role in metabolism, PEPCK could also be altered by additional factors, such as glucose, insulin, glucagon, and epinephrine (Granner and Andreone, 1985). Glucose provoked a time-dependent loss of PEPCK activity (Burlini et al., 1989) and insulin inhibited transcription and accelerated PEPCK mRNA degradation (O’Brien and Granner, 1990). Corticosteroids could elevate both glucose and insulin via increased gluconeogenesis. Therefore, the counter-regulatory biosignal TC could also be explained as insulin. The PEPCK gene promoter contains a cAMP-responsive element (Hanson and Reshef, 1997). Cyclic AMP could also stimulate transcription and inhibit message degradation, thereby increasing PEPCK mRNA (Granner et al., 1986). Such extra complications were not observed in our data; thus, they were not included in our model. Measurements of additional hormones and rates of specific processes would be necessary for further evaluation of CS effects. Overall, our modeling attempted to balance between being parsimonious and being mechanistic. Based on our data and the literature, potential rate-limiting steps of the biological system in vivo were selected as the backbone to provide mathematical description of dynamic changes. Our parameters (such as degradation rate constants) may be a joint estimation of several processes (natural degradation plus control by endogenous substances). Although our estimates may be better representation of in vivo regulation, caution needs to be taken when comparing these values with measurements of specific processes especially in vitro.

A linear transduction model was used to describe the effect of CS in suppressing PEPCK mRNA (eqs. 15 and 16). With the exact mechanism unknown, our transduction model provided the flexibility of capturing the observed phenomenon with mechanistic relevance. This model could be generalized to describe primary and secondary drug effects in regulating the same biological system. As depicted in Fig. 10, drug (Drug) can produce its primary effect by altering the production (kₑ) or disposition (kₑ) of the biomarker (Response). Drug may also affect an endogenous regulator (such as hormones, cytokines, transcription factors), which is simply described by linear transduction. The rate constant (k) reflects the major rate-limiting step producing this additional factor (Biosignal). The drug-altered biosignal regulates the same system causing the secondary effects. Drug and the biosignal may either alter the biomarker in the same manner that could represent multiple mechanisms of drug action, or have counter-regulatory effects that would produce tolerance phenomenon.

In summary, we found induced cAMP and PEPCK in rat liver after MPL treatment. Our results indicate that CS effects might come from both primary source via direct modulation of the dynamic marker, and secondary source via alteration of other controlling factors (such as cAMP). Depending on the relative contributions of different factors/hormones in regulating the biomarker, the secondary response might sometimes predominate. These findings emphasize the importance of studying temporal patterns of drug effect in vivo, which best provides global information. Understanding and quantitation of these effects requires reliable measurements of intermediate biosignals and integration of several known mechanisms. Mechanistic PD models thus provide quantitative description, integration, and additional insights into the biological system and drug effects.

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