

# Activation of cAMP-Dependent Signaling Pathway Induces Mouse Organic Anion Transporting Polypeptide 2 Expression

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## ABSTRACT

Rodent Oatp2 is a hepatic uptake transporter for such compounds as cardiac glycosides. In the present study, we found that fasting resulted in a 2-fold induction of Oatp2 expression in liver of mice. Because the cAMP-protein kinase A (PKA) signaling pathway is activated during fasting, the role of this pathway in Oatp2 induction during fasting was examined. In Hepa-1c1c7 cells, adenylyl cyclase activator forskolin as well as two cellular membrane-permeable cAMP analogs, dibutyl cAMP and 8-bromo-cAMP, induced Oatp2 mRNA expression in a time- and dose-dependent manner. These three chemicals induced reporter gene activity in cells transfected with a luciferase reporter gene construct containing a 7.6-kilobase (kb) 5'-flanking region of mouse Oatp2. Transient transfection of cells with 5'-deletion constructs derived from the 7.6-kb Oatp2 promoter reporter gene construct, as well as 7.6-kb constructs

in which a consensus cAMP response element (CRE) half-site CGTCA (–1808/–1804 bp) was mutated or deleted, confirms that this CRE site was required for the induction of luciferase activity by forskolin. Luciferase activity driven by the Oatp2 promoter containing this CRE site was induced in cells cotransfected with a plasmid encoding the protein kinase A catalytic subunit. Cotransfection of cells with a plasmid encoding the dominant-negative CRE binding protein (CREB) completely abolished the inducibility of the reporter gene activity by forskolin. In conclusion, induction of Oatp2 expression in liver of fasted mice may be caused by activation of the cAMP-dependent signaling pathway, with the CRE site (–1808/–1804) and CREB being the *cis*- and *trans*-acting factors mediating the induction, respectively.

Organic anion transporting polypeptides (Oatps) belong to a family of uptake transporters for structurally diverse organic solutes, including organic anions, cations, and neutral compounds. In rodent species, Oatp2 (also known as Oatp1a4, Slc21a5) is highly expressed in liver, where it is localized to the basolateral membrane domain of hepatocytes (Hagenbuch and Meier, 2003). Oatp2 transports the common OATPs/Oatps substrates, such as estrone-3-sulfate, ouabain, and dehydroepiandrosterone sulfate (Hagenbuch et al., 2000; Hagenbuch and Meier, 2003). Oatp2 also transports thyroid hormones and the cardiac glycoside digoxin. The latter is not a substrate for other OATPs/Oatps except OATP1B3. Hepatic Oatp2 expression can be induced by xenobiotics via the activation of pregnane X receptor and constitutive androstane receptor (CAR) signaling pathways (Hagenbuch et al.,

2001; Guo et al., 2002; Wagner et al., 2005), whereas other liver-predominant OATPs/Oatps are not readily inducible.

cAMP functions as a second messenger in cells in response to such extracellular stimuli as hormones. Binding of cAMP to the regulatory subunit of protein kinase A (PKA) results in the release of the catalytic subunit of PKA. PKA phosphorylates serine 133 of the transcription factor cAMP response element binding protein (CREB). Phosphorylated CREBs form a homodimer that binds to a cAMP response element (CRE) within the promoter region of target genes, controlling gene expression at the transcription level (Parker et al., 1996; Mayr and Montminy, 2001).

In liver, the maintenance of energy homeostasis during starvation and stress is controlled in part by a cAMP-PKA signaling pathway. In addition, several pathways for the metabolism and disposition of endobiotics and xenobiotics are regulated via cAMP-PKA pathways. For example, hepatic expression of heme oxygenase-1 and 5-aminolevulinic synthase, both of which are involved in heme metabolism, is induced via cAMP-PKA signaling pathways (Immenschuh et

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**ABBREVIATIONS:** OATP/Oatp, human/nonhuman organic anion transporting polypeptide; CAR, constitutive androstane receptor; PKA, protein kinase A; CREB, cAMP response element binding protein; CRE, cAMP response element; dbcAMP, di-butyl cAMP; 8-Br-cAMP, 8-bromo cAMP; PPAR, peroxisome proliferator-activated receptor; PCR, polymerase chain reaction; bp, base pair(s); Gapdh, glyceraldehyde-3-phosphate dehydrogenase; kb, kilobase(s); ACREB, dominant-negative CREB; DPM 904, 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-a]-pyrimidine.

al., 1998; Varone et al., 1999). Ziemann et al. (2006) demonstrated that activation of the cAMP-PKA pathway induces multidrug resistance transporter 1b expression in primary rat hepatocytes. In the present study, we found that hepatic expression of Oatp2 was induced in fasted mice. Because cAMP-PKA signaling pathway is activated during fasting, the role of this pathway in Oatp2 induction was examined. Our findings indicate that activation of a cAMP-dependent signaling pathway induces Oatp2 hepatic expression.

## Materials and Methods

**Materials.** Forskolin was obtained from Calbiochem (La Jolla, CA). Di-buteryl cAMP (dbcAMP), 8-bromo cAMP (8-Br-cAMP), and dimethyl sulfoxide were purchased from Sigma (St. Louis, MO).

**Animals.** Male 6-week-old C57BL6 mice were purchased from Charles River Laboratories (Wilmington, MA) and maintained on a 12-h light/dark cycle. After acclimation for 10 days, mice were fasted for 24 h starting at 10:00AM with free access to water, or fasted for 24 h and re-fed for the following 24 h or 48 h. Fed mice were used as control subjects.

Breeding pairs of homozygous peroxisome proliferator-activated receptor (PPAR)  $\alpha$ -null mice on an SV129 background were obtained from Dr. Frank Gonzalez (NCI, Bethesda, MD). Male PPAR $\alpha$ -null mice (3–4 months old) were divided into two groups ( $n = 5$  for each group): a 24-h fasting group and a control feeding group. At the end of experiments, liver was excised and rapidly frozen in liquid nitrogen. Samples were stored at  $-80^{\circ}\text{C}$  until use.

**Total RNA Isolation.** Total RNA was isolated from frozen tissue samples using RNA-Bee reagent (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. After spectrophotometric quantification of RNA concentrations, samples were diluted with diethyl pyrocarbonate-treated water to a final concentration of 1  $\mu\text{g}/\mu\text{l}$ . The integrity of the diluted RNA samples was determined by visual examination of the 18 and 28 S rRNAs separated on 1.2% denaturing agarose gel.

**Cell Culture and Treatment.** Hepa-1c1c7 cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium/Ham's F12 medium (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT), 100  $\mu\text{g}/\text{ml}$  penicillin, and 10  $\mu\text{g}/\text{ml}$  streptomycin (Invitrogen) at  $37^{\circ}\text{C}$  in a humidified incubator maintained at 5%  $\text{CO}_2$ . For treatment, cells were seeded onto 24-well plates at a density of  $5 \times 10^4$  cells/well and allowed to grow to 90 to 95% confluence. Cells were treated with various concentrations of forskolin, dbcAMP, 8-Br-cAMP, or vehicles (dimethyl sulfoxide for forskolin, PBS for dibutyl cAMP and 8-bromo-cAMP) for 4, 8, 18, or 24 h. At the end of treatment, cells were lysed with lysis mixture provided in the branched DNA assay kit (Genospectra, Fremont, CA). Cellular lysate was stored at  $-80^{\circ}\text{C}$  until assay.

**Quantification of Mouse Oatp2 mRNA.** Mouse Oatp2 mRNA was quantified using Quantigene branched DNA signal amplification assay (Genospectra). Branched DNA probe set for mouse Oatp2 was reported previously (Cheng et al., 2005). Branched DNA assay was performed per manufacturer's instructions, using cellular lysate directly or total RNA isolated from liver tissue samples. Oatp2 mRNA expression was normalized to Gapdh mRNA expression. Results were presented as arbitrary units.

**Mouse Oatp2 Promoter Reporter Gene Construct.** A BAC clone (RP24–309B5) containing mouse Oatp2 gene was used as template to produce a 7.6-kb fragment containing the 5'-flanking region of mouse Oatp2 (from  $-7561$  bp to  $+39$  bp), using PCR (forward primer, 5'-CTT CTC GAG GTG AGA AGT CCA CAC ATG AAG GAG-3'; reverse primer, 5'-CTT ACG CGT CAT ATT GTT CCA CCT ATA GGG TTG-3'). Underlined letters indicate XhoI and MluI restriction sites introduced into the primers. The 7.6-kb PCR product was gel-purified and ligated to luciferase reporter gene vector, pGL3-

basic (Promega, Madison, WI). The presence and orientation of the cloned promoter fragment were verified by sequencing into the insert from both the 5' and 3' end of the pGL3 multiple cloning site. Synthesis of PCR primers and DNA sequencing were carried out by the Biotech Support Facility, University of Kansas Medical Center (Kansas City, KS).

**5'-Deleted Mouse Oatp2 Reporter Gene Constructs.** Four 5'-deleted Oatp2 reporter gene constructs (designated as 1.2, 2.6, 3.8, and 5.3 kb) were produced by PCR, using the original 7.6-kb mouse Oatp2 promoter reporter gene construct as a template. The forward primer was 5'-CTT CTC GAG GTG AGA AGT CCA CAC ATG AAG GAG-3'. The reverse primers were 5'-ACG CGT AAT ATC TCA GCT CTT TCT CTT CCT GA-3' (1.2 kb), 5'-ACG CGT TGT GTC TTT GGA CTT GTG TGC G-3' (2.6 kb), 5'-ACG CGT CAG AAT CAG CCT TTG GGA GCT C-3' (3.8 kb), and 5'-ACG CGT CTC TGG CTA GGA CTT CAA GTA CAA TGT-3' (5.3 kb). The PCR products were gel-purified and ligated to pGL3-basic luciferase reporter gene vector. The presence and orientation of the cloned promoter fragment were verified by sequencing into the insert from both the 5' and 3' end of the pGL3 multiple cloning site.

**Site-Directed Mutagenesis.** Site-directed mutagenesis was performed using QuikChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA). The consensus CRE half-site ( $-1808/-1804$  bp, 5'-CGTCA-3') in the 7.6-kb Oatp2 promoter identified by in silico analysis was deleted (designated as  $\Delta 7.6$  kb) or mutated (designated as M7.6 kb). The complementary primers (5'-GAACTCCCCCTG-TGTGCTTTTCAGTTGAGTAAC-3' and 5'-GTTACTCAAC TGAAAA-GCACA\_CAGGGGGAGTTC-3') were used to delete the CRE site. The underscores represent the positions of the deleted CRE site in the primers. The complementary primers (5'-GAACTCCCCCTGTG-GTCGTGTGCTTTTCAGTTGAG-3' and 5'-CTCAACTGAAAAGCA-CACGACCACAGGGGGAGTTC-3') were used to produce AC to GT transition in the CRE site. The mutations and orientation of the cloned promoter fragment were verified by sequencing.

**Transient Transfection and Luciferase Assay.** Cells were seeded onto 24-well plates at a density of  $5 \times 10^4$  cells/well and transfected using Lipofectamine 2000 reagent (Invitrogen) when reaching approximately 50 to 80% confluence. Where indicated, cells were transfected with mouse Oatp2 reporter gene constructs (500 ng) and pRL-TK vector encoding *Renilla reniformis* luciferase (50 ng), or reporter gene constructs along with expression plasmid encoding wild-type or mutant PKA catalytic subunit (50 ng) (Orellana and McKnight, 1992), as well as dominant-negative CREB (ACREB) (Ahn et al., 1998) or its empty vector CMV200 (2 and 10 ng). After transient transfection for 18 h, cells were treated with forskolin (5  $\mu\text{M}$ ) or cAMP analogs (0.5 mM) for 24 h. Cells were lysed and dual luciferase assays were performed according to the manufacturer's instructions (Promega).

## Results

**Oatp2 mRNA in Liver of Fasted Mice.** As shown in Fig. 1A, hepatic expression of Oatp2 was increased approximately 2-fold in mice that were fasted for 24 h compared with fed mice. The magnitude of Oatp2 induction in mice that were re-fed for 24 h after a 24-h fasting was similar to that of mice that were fasted for 24 h only. Oatp2 induction was attenuated in mice that were re-fed for 48 h after a 24-h fasting.

A 2-fold induction of hepatic Oatp2 expression was also observed in PPAR $\alpha$ -null mice that were fasted for 24 h (Fig. 1B). Oatp2 basal expression in fed PPAR $\alpha$ -null mice was 6-fold higher than that in fed C57/BL6 mice. No differences in the hepatic expression of Gapdh, the house-keeping gene to which Oatp2 mRNA was normalized, were observed between the fed C57BL6 and the fed PPAR $\alpha$ -null mice ( $7294 \pm 545$  versus  $7856 \pm 565$  relative light units/10  $\mu\text{g}$  of total RNA).

**Oatp2 Induction in Hepa1c1c7 Cells.** Hepa-1c1c7 cells were treated with various concentrations of the adenylyl cyclase activator, forskolin (0.5, 1, 2, and 10  $\mu$ M), or two membrane-permeable analogs of cAMP, dbcAMP and 8-Br-cAMP (0.01, 0.1, 0.5, and 1 mM) for 18 h. Forskolin, dbcAMP, and 8-Br-cAMP dose dependently induced Oatp2 expression in Hepa-1c1c7 cells (Fig. 2A). The maximal induction was approximately 4-fold.

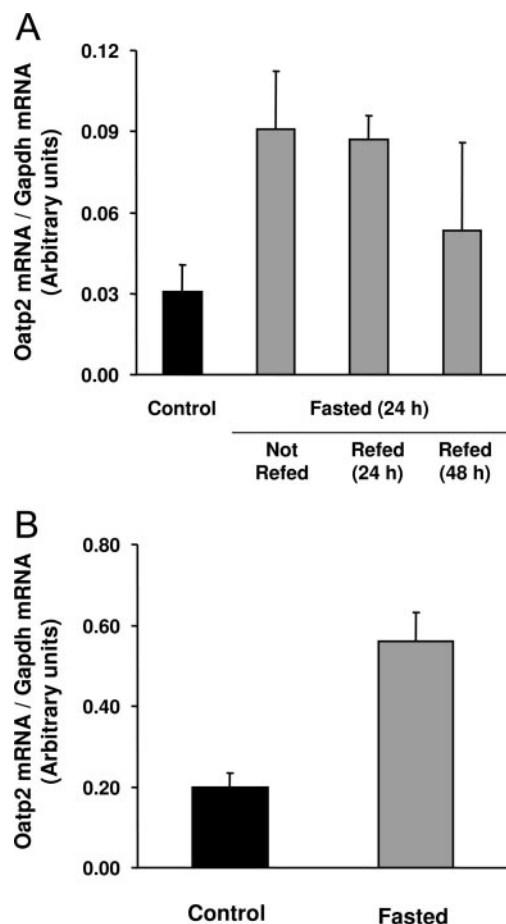
Hepa-1c1c7 cells were also treated with forskolin (5  $\mu$ M), dbcAMP or 8-Br-cAMP (0.5 mM) for 4, 8, 18, or 24 h. As shown in Fig. 2B, Oatp2 expression in Hepa-1c1c7 cells was induced in a time-dependent manner. Maximal induction was observed after 18 h of treatment.

**Induction of Luciferase Activity Driven by Oatp2 Promoter.** A luciferase reporter gene construct containing a 7.6-kb promoter region of mouse Oatp2 gene (from -7561 bp to +39 bp) was generated as described under *Materials and Methods*. To determine whether the 7.6-kb Oatp2 promoter region contains the DNA sequences that mediate induction of Oatp2, Hepa-1c1c7 cells were transfected with this Oatp2 reporter gene construct. Then, transfected cells were treated with forskolin (5  $\mu$ M), dbcAMP (0.5 mM), 8-Br-cAMP (0.5 mM), or vehicle. Luciferase activity driven by the 7.6-kb Oatp2 promoter was induced by 4- to 6-fold after treatment (Fig. 3). These results indicate that the regulatory DNA

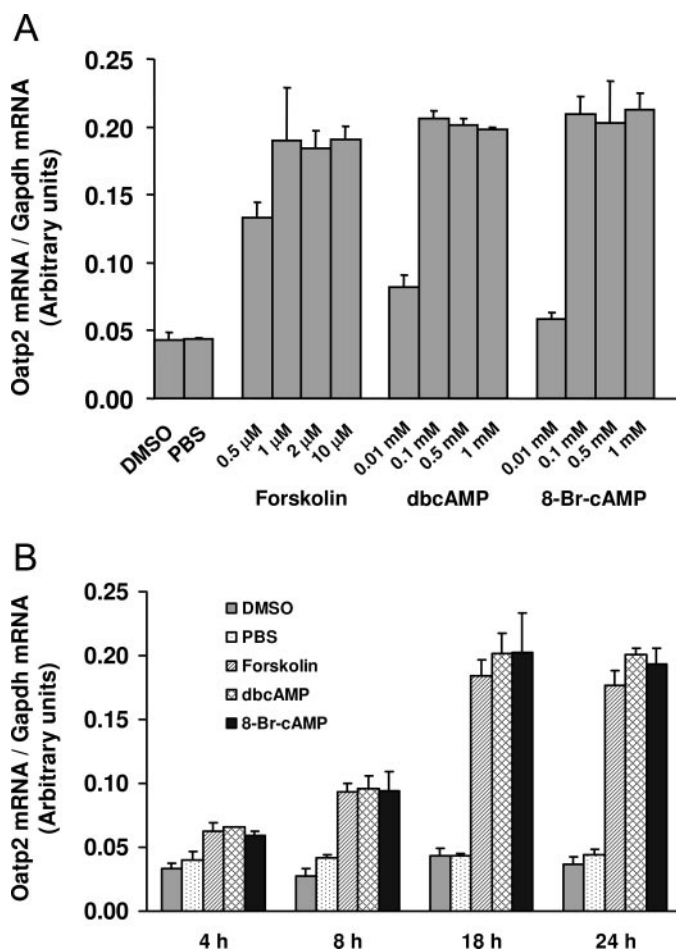
sequences that mediate Oatp2 induction are located within the 7.6-kb promoter.

**Oatp2 Promoter Deletion Analysis.** To further determine the location of the DNA sequences that mediate Oatp2 induction, a series of 5'-deletion constructs of the 7.6-kb Oatp2 reporter gene construct were generated as described under *Materials and Methods*. The size of the deletion constructs ranges from 1.2 to 5.3 kb. Hepa-1c1c7 cells were transfected with the reporter gene constructs of different sizes. Transfected cells were treated with forskolin for 18 h. Forskolin did not affect the reporter gene activity driven by the 1.2-kb Oatp2 promoter (Fig. 4A). Luciferase activity driven by the other Oatp2 promoters (2.6, 3.8, 5.3, and 7.6 kb) was induced by forskolin.

Protein kinase A is a key component of the cAMP-dependent pathway. The PKA catalytic subunit phosphorylates the transcription factor CREB in response to elevated intracellular cAMP levels. Therefore, the effects of PKA catalytic subunit on gene expression were determined to further establish the role of cAMP-dependent pathway in the induction



**Fig. 1.** Oatp2 mRNA expression in liver of fasted mice. A, C57BL6 mice. Groups of mice were fasted for 24 h, or fasted for 24 h followed by re-feeding for 24 and 48 h, respectively. Fed mice were used as control subjects. B, PPAR $\alpha$ -null mice. Mice were fasted for 24 h. Fed mice were used as control subjects. Data are expressed as means  $\pm$  S.D. ( $n = 5$ ).

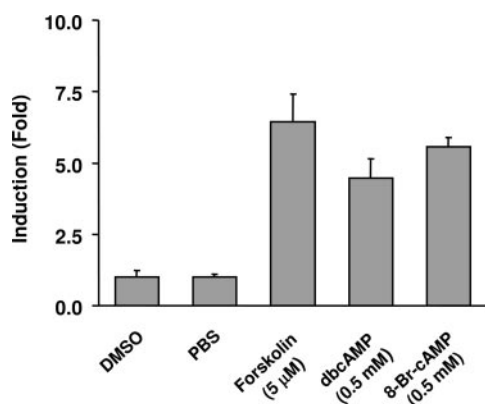


**Fig. 2.** Induction of mouse Oatp2 mRNA expression in Hepa-1c1c7 cells treated with the adenylyl cyclase activator forskolin and two membrane-permeable analogs of cAMP, dbcAMP and 8-Br-cAMP. A, dose-response study. Hepa-1c1c7 cells were treated with forskolin (0.5, 1, 2, and 10  $\mu$ M), dbcAMP or 8-Br-cAMP (0.01, 0.1, 0.5, and 1 mM) for 18 h. B, time-response study. Hepa-1c1c7 cells were treated with forskolin (5  $\mu$ M), dbcAMP or 8-Br-cAMP (0.5 mM) for 4, 8, 18, or 24 h. Oatp2 mRNA levels were quantified using branched DNA assays. Oatp2 mRNA expression was normalized to Gapdh mRNA expression. Results are expressed as arbitrary units (means  $\pm$  S.D. of three separate experiments performed in triplicate).

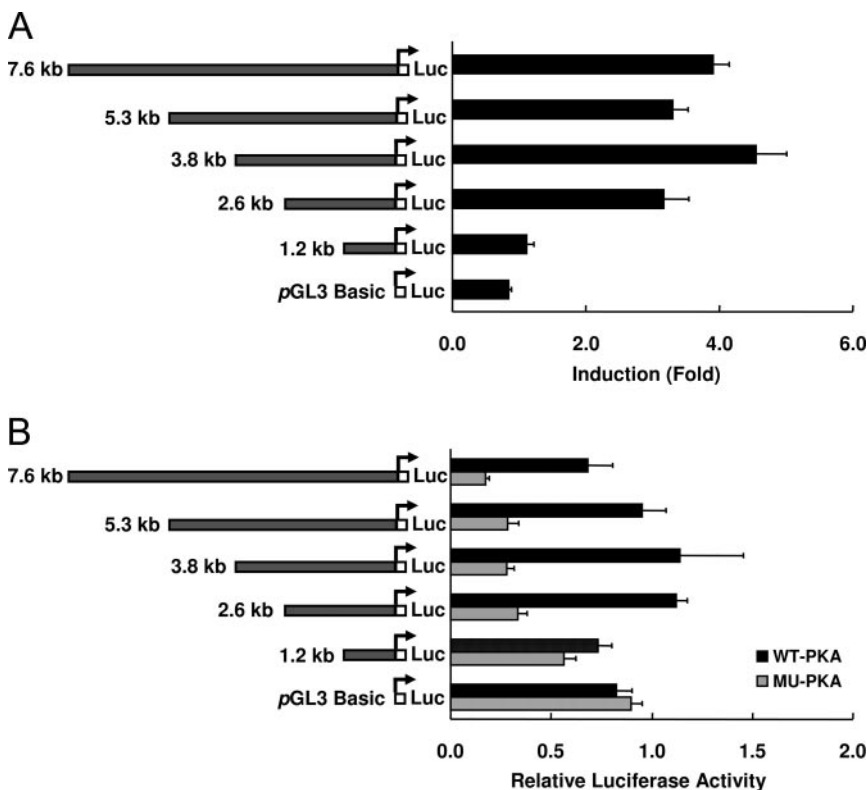


of Oatp2. Hepa-1c1c7 cells were cotransfected with expression plasmid encoding PKA catalytic subunit and the Oatp2 promoter reporter gene constructs. As shown in Fig. 4B, cotransfection of wild-type PKA expression plasmid did not induce luciferase activity driven by the 1.2-kb promoter. However, cotransfection of wild-type PKA expression plasmid caused a 3-fold induction of luciferase activity driven by 2.6-, 3.8-, 5.3- and 7.6-kb Oatp2 promoter, respectively. The results from this experiment support our hypothesis that a cAMP-dependent pathway plays a role in Oatp2 induction.

**Reporter Assay Using Oatp2 Reporter Gene Constructs in Which a Putative CRE Is Deleted or Mutated.** In silico analysis of the Oatp2 promoter identified a consensus CRE half-site, CGTCA, located at approximately



**Fig. 3.** Induction of mouse Oatp2 promoter-driven luciferase activity. Hepa-1c1c7 cells were transfected with the 7.6-kb Oatp2 promoter firefly luciferase construct and pRL-TK *R. reniformis* luciferase construct. Transfected cells were treated with forskolin (5 μM), dbcAMP (0.5 mM), 8-Br-cAMP (0.5 mM), or vehicles for 18 h. Dual luciferase assays were performed. Results are expressed as -fold induction (means ± S.D. of three separate experiments performed in triplicate).



**Fig. 4.** Oatp2 promoter deletion analysis. A, forskolin treatment. Hepa-1C1c7 cells were transfected with the reporter gene constructs of different sizes. Transfected cells were treated with forskolin (5 μM). Results were expressed as -fold induction (means ± S.D. of three separate experiments performed in triplicate). B, cotransfection of PKA catalytic subunit. Cells were cotransfected with expression plasmid encoding PKA catalytic subunit and the Oatp2 promoter reporter gene constructs. Luciferase activity from cells cotransfected with plasmid encoding the wild-type PKA catalytic subunit was compared with that from cells cotransfected with plasmid encoding the mutant PKA without any catalytic activity. Results are expressed as relative luciferase activity (means ± S.D. of three separate experiments performed in triplicate).

1.8 kb upstream from the transcription starting site. This element is not present in the 1.2-kb promoter construct but is present in the other larger constructs. Based on the results of the promoter deletion study (Fig. 4), it is possible that this consensus CRE half-site may be involved in Oatp2 induction by forskolin.

To further determine whether this CRE half-site is needed for the induction of gene expression, transient reporter gene assays were performed using Oatp2 promoter reporter gene constructs in which this putative CRE was deleted or mutated. As shown in Fig. 5A, deletion or mutation almost completely abolished the inducibility of luciferase activity after forskolin treatment. Deletion or mutation of the same element also abolished the inducibility of luciferase activity in cells that were cotransfected with expression plasmid encoding wild-type PKA catalytic subunit (Fig. 5B).

**Effects of ACREB on Inducibility of Luciferase Activity Driven by Oatp2 Promoter.** To establish CREB as the *trans*-acting factor that mediates Oatp2 induction, transient reporter gene assays were performed using the dominant-negative CREB described previously. This dominant-negative CREB can dimerize with the wild-type CREB and prevents the wild-type CREB from binding to DNA. It is clear that cotransfection of the dominant-negative CREB (2 and 10 ng) abolished the inducibility of luciferase activity after forskolin treatment (Fig. 6). The results strongly indicate that CREB is the *trans*-acting factor mediating Oatp2 induction.

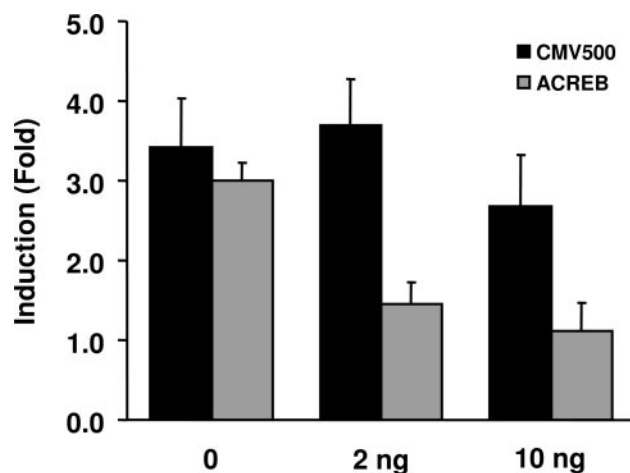
## Discussion

In this study, a 2-fold induction of hepatic Oatp2 in fasted mice was observed (Fig. 1). Further experiments using Hepa-1c1c7 cell line provided several lines of evidence indicating that activation of cAMP-PKA pathway contributes to this

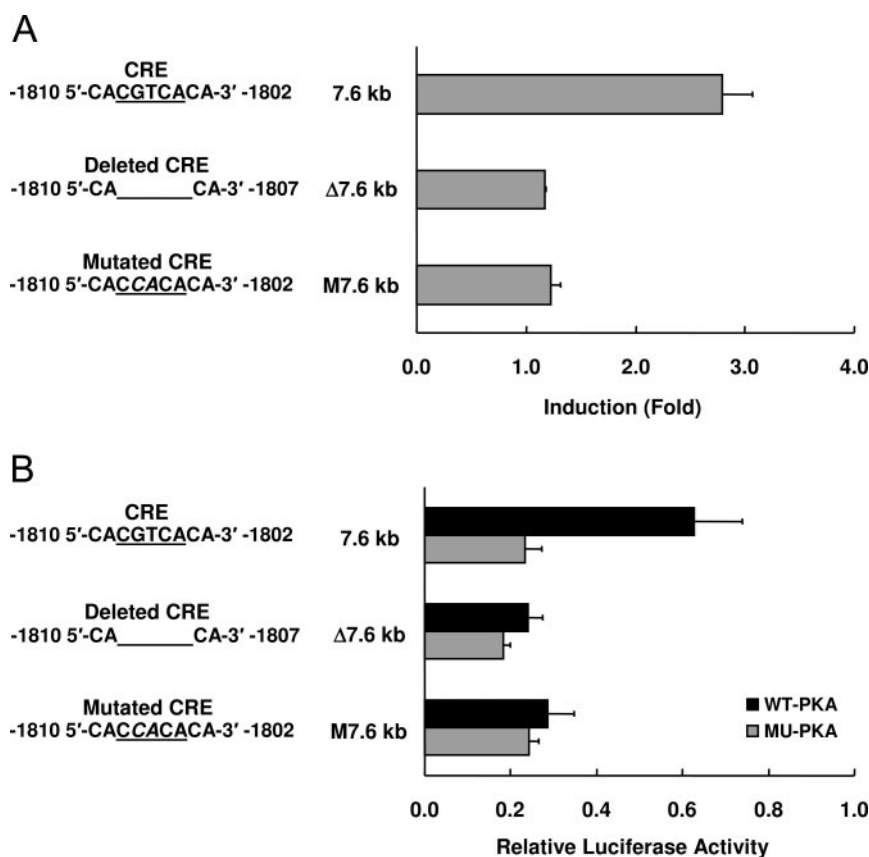
induction: 1) treatment of cells with forskolin and two cAMP analogs dose- and time-dependently induced Oatp2 mRNA, 2) forskolin treatment induced luciferase activity driven by a 7.6-kb Oatp2 promoter, 3) the location of the DNA sequence mediating the induction was narrowed down to approximately -1200 to -2600 bp by deletion analysis of a 7.6-kb Oatp2 promoter reporter gene construct, 4) deletion and mutation of the consensus CRE half-site, CGTCA, located at -1804 bp, abolished the inducibility of luciferase activity, and 5) cotransfection of a dominant-negative CREB abolished the inducibility of luciferase activity (Figs. 2–6). CREB regulates gene expression by binding to a CRE that exists either as a palindrome (TGACGTCA) or half-site (CGTCA/TGACG) (Mayr and Montminy, 2001). According to a recent study (Zhang et al., 2005), most conserved CREs found in gene promoter regions are located within 200 bp of the transcription start site. It is noteworthy that in this study, a functional CRE half-site was identified at -1804 bp. Taken together, the consensus CRE half-site at -1808/-1804 bp and CREB seem to be the *cis*- and *trans*-acting factors, respectively, mediating the induction of Oatp2.

During fasting, the body relies more on fatty acids and ketones than on glucose for energy production. This adaptive metabolic response is controlled in part by the nuclear receptor PPAR $\alpha$ . Kok et al. (2003) reported that PPAR $\alpha$  activation leads to induction of multidrug resistance transporter 2 in liver of fasted mice. Moreover, phosphorylation of PPAR $\alpha$  by PKA has been shown to increase its activity (Lazennec et al., 2000), suggesting that PPAR $\alpha$  pathway, to some extent, functions downstream of the PKA pathway. Therefore, the role of PPAR $\alpha$  in Oatp2 induction during fasting was examined using PPAR $\alpha$ -null mice. Our results indicated that Oatp2

induction is independent of PPAR $\alpha$ , although loss of PPAR $\alpha$  seems to lead to elevated basal expression of Oatp2 in liver of PPAR $\alpha$ -null mice (Fig. 1B). Maglich et al. (2004) reported that fasting induces the expression of CAR target genes such as *Cyp2b10* and *Ugt1a1* in a receptor-dependent manner. These authors proposed that CAR could be activated without ligand binding in response to metabolic and nutritional stress. Future study using CAR-null mice will help to elucidate the inter-relationship between CAR and cAMP-PKA



**Fig. 6.** Transient transfection of the dominant-negative CREB abolishes the inducibility of luciferase activity driven by Oatp2 promoter. Hepa-1c1c7 cells were cotransfected with the 7.6-kb Oatp2 reporter gene construct and plasmid encoding ACREB or empty vector CMV200. Cotransfected cells were treated with forskolin (5  $\mu$ M). Results are expressed as -fold induction (means  $\pm$  S.D. of three separate experiments performed in triplicate).



**Fig. 5.** Deletion and mutation analysis of a putative CRE located at 1.8 kb upstream from the transcription starting site of mouse Oatp2. **A**, forskolin treatment. Hepa-1c1c7 cells were transfected with the 7.6-kb Oatp2 reporter gene construct in which the putative CRE was deleted or mutated. Transfected cells were treated with forskolin (5  $\mu$ M). Results are expressed as -fold induction (means  $\pm$  S.D. of three separate experiments performed in triplicate). **B**, cotransfection of PKA catalytic subunit. Cells were cotransfected with expression plasmid encoding PKA catalytic subunit and the mutated reporter gene constructs. Luciferase activity from cells cotransfected with plasmid encoding the wild-type PKA catalytic subunit was compared with that from cells cotransfected with plasmid encoding the mutant PKA without any catalytic activity. Results are expressed as relative luciferase activity (means  $\pm$  S.D. of three separate experiments performed in triplicate).

signal pathway in the regulation of Oatp2 and other genes during fasting.

Caloric restriction has been shown to decrease serum thyroid hormone levels in mice and human subjects (Rosenbaum et al., 2000; Maglich et al., 2004). Maglich et al. (2004) attributed this phenomenon to increased hepatic metabolism of thyroid hormones via glucuronidation and sulfation pathways. Wong et al. (2005) reported that induction of hepatic Oatp2 expression in rats treated with a compound known as DPM 904 is associated with increased hepatobiliary clearance of unconjugated thyroid hormones and decreased serum thyroid hormone concentrations. Therefore, it is possible that increased hepatic expression of Oatp2 may be another factor contributing to the accelerated thyroid hormone elimination during caloric restriction. This speculation is in accordance with our observation that Oatp2 was induced in liver of fasted mice (Fig. 1). Besides Oatp2 and other OATPs/Oatps, other types of transporters, such as monocarboxylate transporter 8 and 10, have been shown to transport thyroid hormones according to results from in vitro transport assays (Friesema et al., 2005). However, a correlation between altered expression of thyroid hormone transporters and changes of serum hormone levels has not been reported previously, making it difficult to ascertain the in vivo importance of these various transporters in the disposition of thyroid hormones. Our current findings, together with those of others, suggest that Oatp2 may play a role in the hepatic elimination of thyroid hormones, and that this process is induced via cAMP-PKA pathway during starvation and may cause increased elimination of thyroid hormones and decreased body energy expenditure.

It is well known that fasting often increases susceptibility to chemical-induced liver injury. In some cases, the increase in susceptibility in fasted animals can be attributed to either induction of P450 isozymes or depletion of hepatic glutathione levels. In light of our findings that hepatic Oatp2 expression was induced in fasted mice, it is possible that hepatic induction of Oatp2 during fasting increases hepatic uptake of drugs and hepatotoxicants that are Oatp2 substrates, contributing to the increase in susceptibility to liver toxicity.

In conclusion, we found that fasting resulted in induction of hepatic expression of Oatp2 in mouse. Activation of cAMP-PKA pathway seems to be the underlying mechanism for the induction.

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