

MODULATION OF P-GLYCOPROTEIN EXPRESSION IN HYPERTHYROID RAT TISSUES

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ABBREVIATIONS:

Pgp, P-glycoprotein; T₄, L-Thyroxine; bsep, bile salt export pump; PCR, polymerase chain reaction.

ABSTRACT

P-glycoprotein (Pgp) is expressed in various normal tissues and plays an important role in drug absorption and disposition. In addition, it is supposed that alterations in the expression levels of Pgp are involved in the inter- and intraindividual variability of pharmacokinetics of many drugs. Since pharmacokinetic properties of various drugs are altered in patients with thyroid disorders, we examined the expression of Pgp and *mdr1a/1b* mRNA in the kidney, liver, jejunum and ileum from euthyroid and hyperthyroid rats. Western blot analysis revealed that Pgp expression was markedly increased in the kidney and liver of hyperthyroid rats. In contrast, it was slightly increased in the jejunum and ileum. *Mdr1a/1b* mRNA levels were significantly increased in the kidney of hyperthyroid rats. However, those were not increased in the liver as well as in the jejunum and ileum of hyperthyroid rats. Expression levels of bile salt export pump and *mdr2* mRNA were also unchanged in hyperthyroid rat liver. Taken together, these findings suggest that thyroid hormone induces Pgp expression in a tissue-selective manner and the modulation of *mdr1a/1b* mRNA expression in the hyperthyroid state varied among tissues.

P-glycoprotein (Pgp) is expressed in various tissues such as brain, liver, kidney and intestine (Brady et al., 2002; Cordon-Cardo et al., 1990) and plays an important role in defining the pharmacokinetics of many drugs. Pgp functions as a drug efflux pump and exports hydrophobic, bulky drugs such as anticancer agents, cardiac glycosides, β -blockers, calcium channel blockers and immunosuppressants. Since Pgp has a broad substrate recognition, the concomitant administration of drugs often causes drug interactions by inhibiting Pgp-mediated transport (Yu, 1999). For example, inhibition of digoxin transport in cultured epithelial cell lines expressing Pgp by various drugs such as quinidine (Fromm et al., 1999; Tanigawara et al., 1992), verapamil (Tanigawara et al., 1992) and cyclosporin A (Okamura et al., 1993) has been reported. We have also demonstrated that the renal clearance of digoxin was decreased in patients receiving a concomitant administration of clarithromycin and accordingly the plasma concentration of digoxin was increased (Wakasugi et al., 1998). The mechanism of this interaction was explained by the inhibition of Pgp-mediated tubular secretion of digoxin. On the other hand, recent studies have demonstrated that changes in the expression levels of Pgp affect the pharmacokinetic properties of Pgp substrates. Greiner *et al.* (1999) reported that rifampin administration induced Pgp expression in the small intestine and reduced the plasma concentration of orally administered digoxin, suggesting that alterations in the expression levels of Pgp are closely involved in the inter- and intraindividual variability of pharmacokinetics of Pgp substrates.

Thyroid hormone is secreted from the thyroid gland to maintain normal growth and development, normal body temperature, and normal energy levels. Most of its effects appear to be mediated by the activation of nuclear receptors that lead to increased expression of mRNA and subsequent protein synthesis. Disorders of the thyroid gland are among the most common endocrine disorders, and are known as hyperthyroidism and hypothyroidism. It was reported that pharmacokinetic properties of various drugs were altered in patients with thyroid disorders (O'Connor and Feely, 1987; Shenfield, 1981). As for Pgp substrates, plasma concentrations of digoxin were decreased in patients with hyperthyroidism as compared with euthyroid patients. Such an altered pharmacokinetics of digoxin in hyperthyroid patients has been explained by an increase in renal clearance (Lawrence et al., 1977) and volume of distribution (Shenfield et al., 1977; Shenfield, 1981). The reason for the increased renal clearance of digoxin is considered to be a facilitation of tubular secretion (Bonelli et al., 1978), however, the mechanisms underlying the altered pharmacokinetics of digoxin in thyroid disease have not been fully elucidated.

Previous studies have shown that thyroid hormone regulates the expression levels of various membrane transporters such as the fructose transporter GLUT5 (Matosin-Matekalo et al., 1999), the peptide transporter PEPT1 (Ashida et al., 2002), $\text{Na}^+\text{-K}^+\text{-ATPase}$ (Giannella et al., 1993) and the Na^+/H^+ exchanger NHE1 (Li et al., 2002). Therefore, we hypothesized that the alteration in the plasma concentration of digoxin in patients with thyroid disorders might be due to changes in Pgp expression by thyroid hormone. In the present study, to elucidate the influence of a hyperthyroid state on the expression of Pgp in various tissues, we investigated Pgp and *mdr1a/1b* mRNA levels in liver, kidney, jejunum and ileum from euthyroid and hyperthyroid rats.

Materials and Methods

Materials. L-Thyroxine (T₄) was purchased from Sigma-Aldrich Co. (St. Louis, MO). Monoclonal antibody C219 and anti-villin polyclonal antibody were from CIS Bio International (Gif-sur-Yvette, France) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), respectively. All other chemicals used were of the highest purity available.

Animals. The animal experiments were performed in accordance with the Guideline for Animal Experiments of Kyoto University. Eight-week-old male Wistar rats were housed in a temperature- and humidity-controlled room and fed rat chow *ad libitum*. Hyperthyroidism was induced by adding T₄ (12 mg/L) to the drinking water for 21 days as previously described (Ashida et al., 2004). After treatment, the kidney, liver, jejunum and ileum were excised. Blood was also collected for measurement of plasma level of T₃. Plasma T₃ levels in euthyroid and hyperthyroid rats were measured by an Enzyme Immuno Assay method (IMx, Dainabot, Tokyo, Japan). Plasma T₃ levels in euthyroid and hyperthyroid rats were 0.32 ± 0.02 and 2.40 ± 0.18 ng/ml, respectively (mean ± SE, n=6). In addition, hyperthyroid rats lost an average of 0.055 kg in response to T₄ treatment (data not shown).

Western Blot Analysis. Isolation of crude plasma membrane fractions from each tissue and Western blot analysis were performed as described previously (Ogihara et al., 1996). Monoclonal antibody C219 and anti-villin polyclonal antibody were used as primary antibodies.

RNA Isolation, Semiquantitative Reverse Transcription-Polymerase Chain Reaction (PCR) and Competitive PCR. RNA isolation, reverse transcription and competitive PCR procedures were performed as described previously (Masuda et al., 2000) with some modifications. The specific primer sets (5 μM) used were as follows: for rat *mdr1a* primers 5'-GATGGAATTGATAATGTGGACA-3' and 5'-AAGGATCAGGAACAATAAAA-3', for rat *mdr1b* primers 5'-GAAATAATGCTTATGAATCCCCAAA G-3' and 5'-GGTTTCATGGTCGTCGTCTCTTGA-3' (Zhang et al., 1996), for rat bile salt export pump (*bsep*) primers 5'-GAGGTTACTTAATAGCCTACG-3' and 5'-CATCTATCATCACAGTTCCC-3', and for rat *mdr2* primers 5'-AAGAATTTGAAGTTGAGCTAAGTGA-3' and 5'-TGGTTTCCACATCCAGC CTAT-3'. For the detection of *mdr1a* and *mdr1b*, semi-logarithmic serial dilutions of mimic competitor DNA from 50 to 0.01 amol were added.

Statistical Analysis. Data were analyzed statistically using the nonpaired t-test. Probability values of less than 5 percent were considered significant.

Results and Discussion

To investigate the effect of thyroid hormone on the expression of Pgp, we examined the Pgp expression by Western blotting. As shown in Fig. 1, the expression of Pgp was remarkably increased in crude membranes of hyperthyroid rat kidney and liver (1.9-fold and 2.3-fold, respectively) as compared with those of euthyroid rats. In contrast, hyperthyroidism caused a slight increase in the expression of Pgp in rat jejunum and ileum (1.1-fold and 1.4-fold, respectively). These results suggest that the differences in the behavior of thyroid hormone in each tissue are involved in tissue selectivity of Pgp induction. Similarly, it was reported that MDR1 mRNA was elevated in a dexamethasone-treated human hepatoma cell line but not in a non-hepatoma cell line suggesting that the hormonal regulation of *mdr* gene expression is gene- and cell type-specific (Zhao et al., 1993). Their report supports our results suggesting that the regulation of Pgp expression by thyroid hormone was possibly tissue-specific.

We then examined the expression of *mdr1a* and *mdr1b* mRNA using competitive PCR in each tissue. As shown in Fig. 2, the expression of both *mdr1a* and *mdr1b* mRNA was significantly increased in hyperthyroid rat kidney as compared with the control. Since the action of thyroid hormone appears to be mediated by the activation of nuclear receptors which leads to increased levels of mRNA and subsequent protein synthesis (Ribeiro et al., 1995), it is likely that the induction of Pgp expression in the hyperthyroid kidney is mediated by the increased transcription of *mdr1a/1b* mRNA. On the other hand, hyperthyroidism caused a significant decrease in the expression of *mdr1b* mRNA in the liver, but did not affect the expression of *mdr1a* mRNA. Since it was reported that C219 antibody reacted with not only Pgp but bsep and *mdr2* (Childs et al., 1995) and bsep and *mdr2* are expressed in the liver, we examined whether the immunoreactive protein bands obtained using C219 antibody reflected the induction of these transporters in hyperthyroid liver. As shown in Fig. 3, however, the expression of bsep and *mdr2* mRNA did not change in hyperthyroid rat liver. Although the precise mechanism is not clear at this stage, thyroid hormone may regulate Pgp expression via non-transcriptional control in the liver. Further studies are needed to elucidate the regulation of Pgp and MDR1 by thyroid hormone in the liver.

Siegmund et al. (2002) examined the effect of levothyroxine administration on human intestinal Pgp expression. They demonstrated that duodenal MDR1 mRNA expression and immunoreactive Pgp were increased by levothyroxine administration, although the increase in MDR1 mRNA was not significant. However, changes in the expression of Pgp were not associated with major alterations in the pharmacokinetics of talinolol, a substrate of Pgp. In addition, the effect of thyroid hormone on the expression of Pgp in other tissues was not elucidated. In the present study, we observed a significant increase in Pgp expression in the kidney and liver (Fig. 2) and a slight increase in the intestine (Fig. 4) in hyperthyroid rats. Our results suggest that the decrease in the serum concentration of digoxin in hyperthyroidism is attributable to the increased expression of Pgp in the kidney and/or liver. Further studies are needed to assess the pharmacokinetics of Pgp substrates in hyperthyroid rats.

For obvious ethical reasons, Siegmund et al. (2002) administered levothyroxine in doses that do not cause thyrotoxicosis. In the present study, the expression of Pgp was only slightly increased in rat intestinal tissues, though we used hyperthyroid rats. It is known that the gene encoding Pgp differs between humans and rats. Pgp is encoded by *MDR1* in humans and by *mdr1a* and *mdr1b* in rats. Therefore, it is supposed that the mechanisms by which thyroid hormone induces Pgp expression differ between species and Pgp expression in human intestinal tissues may increase dramatically under thyrotoxicosis.

In conclusion, thyroid hormone induces Pgp expression in a tissue-selective manner. In addition, the modulation of *mdr1a/1b* mRNA expression in the hyperthyroid state varied among tissues. These results provide useful information for elucidating the drug interaction and pharmacokinetic variability in thyrotoxicosis.

References

- Ashida K, Katsura T, Motohashi H, Saito H, and Inui K (2002) Thyroid hormone regulates the activity and expression of the peptide transporter PEPT1 in Caco-2 cells. *Am J Physiol Gastrointest Liver Physiol* **282**:G617-G623.
- Ashida K, Katsura T, Saito H, and Inui K (2004) Decreased activity and expression of intestinal oligopeptide transporter PEPT1 in rats with hyperthyroidism *in vivo*. *Pharm Res* **21**:969-975.
- Bonelli J, Haydl H, Hrudy K, and Kaik G (1978) The pharmacokinetics of digoxin in patients with manifest hyperthyroidism and after normalization of thyroid function. *Int J Clin Pharmacol Biopharm* **16**:302-306.
- Brady JM, Cherrington NJ, Hartley DP, Buist SC, Li N, and Klaassen CD (2002) Tissue distribution and chemical induction of multiple drug resistance genes in rats. *Drug Metab Dispos* **30**:838-844.
- Childs S, Yeh RL, Georges E, and Ling V (1995) Identification of sister gene to P-glycoprotein. *Cancer Res* **55**:2029-2034.
- Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, and Melamed MR (1990) Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem* **38**:1277-1287.
- Fromm MF, Kim RB, Stein CM, Wilkinson GR, and Roden DM (1999) Inhibition of P-glycoprotein-mediated drug transport. A unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* **99**:552-557.
- Giannella RA, Orłowski J, Jump ML, and Lingrel JB (1993) Na⁺-K⁺-ATPase gene expression in rat intestine and Caco-2 cells: response to thyroid hormone. *Am J Physiol* **265**:G775-G782.
- Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, and Kroemer HK (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* **104**:147-153.
- Lawrence JR, Sumner DJ, Kalk WJ, Ratcliffe WA, Whiting B, Gray K, and Lindsay M (1977) Digoxin kinetics in patients with thyroid dysfunction. *Clin Pharmacol Ther* **22**:7-13.
- Li X, Misik AJ, Rieder CV, Solaro RJ, Lowen A, and Fliegel L (2002) Thyroid hormone receptor α_1 regulates expression of the Na⁺/H⁺ exchanger (NHE1). *J Biol Chem* **277**:28656-28662.
- Marzolini C, Paus E, Buclin T, and Kim RB (2004) Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* **75**:13-33.
- Masuda S, Uemoto S, Hashida T, Inomata Y, Tanaka K, and Inui K (2000) Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. *Clin Pharmacol Ther* **68**:98-103.
- Matosin-Matekalo M, Mesonero JE, Laroche TJ, Lacasa M, and Brot-Laroche E (1999) Glucose and thyroid hormone co-regulate the expression of the intestinal fructose transporter GLUT5. *Biochem J* **339**:233-239.
- O'Connor P and Feely J (1987) Clinical pharmacokinetics and endocrine disorders – therapeutic implications. *Clin Pharmacokinet* **13**:345-364.
- Ogihara H, Saito H, Shin BC, Terada T, Takenoshita S, Nagamachi Y, Inui K, and Takata K (1996)

- Immuno-localization of H⁺/peptide cotransporter in rat digestive tract. *Biochem Biophys Res Commun* **220**:848-852.
- Okamura N, Hirai M, Tanigawara Y, Tanaka K, Yasuhara M, Ueda K, Komano T, and Hori R (1993) Digoxin-cyclosporin A interaction: modulation of the multidrug transporter P-glycoprotein in the kidney. *J Pharmacol Exp Ther* **266**:1614-1619.
- Ribeiro RCJ, Apriletti JW, West BL, Wagner RL, Fletterick RJ, Schaufele F, and Baxter JD (1995) The molecular biology of thyroid hormone action. *Ann NY Acad Sci* **758**:366-389.
- Shenfield GM, Thompson J, and Horn DB (1977) Plasma and urinary digoxin in thyroid dysfunction. *Eur J Clin Pharmacol* **12**:437-443.
- Shenfield GM (1981) Influence of thyroid dysfunction on drug pharmacokinetics. *Clin Pharmacokinet* **6**:275-297.
- Siegmund W, Altmannsberger S, Paneitz A, Hecker U, Zschiesche M, Franke G, Meng W, Warzok R, Schroeder E, Sperker B, Terhaag B, Cascorbi I, and Kroemer HK (2002) Effect of levothyroxine administration on intestinal P-glycoprotein expression: consequences for drug disposition. *Clin Pharmacol Ther* **72**:256-264.
- Tanigawara Y, Okamura N, Hirai M, Yasuhara M, Ueda K, Kioka N, Komano T, and Hori R (1992) Transport of digoxin by human P-glycoprotein expressed in a porcine kidney epithelial cell line (LLC-PK₁). *J Pharmacol Exp Ther* **263**:840-845.
- Wakasugi H, Yano I, Ito T, Hashida T, Futami T, Nohara R, Sasayama S, and Inui K (1998) Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. *Clin Pharmacol Ther* **64**:123-128.
- Yu DK (1999) The contribution of P-glycoprotein to pharmacokinetic drug-drug interactions. *J Clin Pharmacol* **39**:1203-1211.
- Zhang F, Riley J, and Gant TW (1996) Use of internally controlled reverse transcriptase-polymerase chain reaction for absolute quantitation of individual multidrug resistant gene transcripts in tissue samples. *Electrophoresis* **17**:255-260.
- Zhao JY, Ikeguchi M, Eckersberg T, and Kuo MT (1993) Modulation of multidrug resistance gene expression by dexamethasone in cultured hepatoma cells. *Endocrinology* **133**:521-528.

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Figure legends

FIG.1. Western blot analysis of the crude membranes from rat tissues for Pgp. Upper panel: immunoblotting of crude membranes from kidney, liver, jejunum and ileum of hyperthyroid rats (T_4) or euthyroid rats (control). Lower panel: densitometric quantification of Pgp. Each column represents the mean \pm SE of 3~7 rats. * $p < 0.05$, significantly different from control.

FIG. 2. Effect of hyperthyroidism on the expression of mdr1a (A) and mdr1b (B) mRNA in rat kidney and liver. Upper panel: typical results of agarose gel electrophoresis of the PCR products from each tissue of hyperthyroid rats (T_4) or euthyroid rats (control). Eight points were selected with serial dilutions of mdr1a/1b mimic competitor cDNA from 0.01 to 25 amol. Lower panel: densitometric quantification of mdr1a and mdr1b mRNA. Each column represents the mean \pm SE of 4 rats. * $p < 0.05$, significantly different from control.

FIG. 3. Effect of hyperthyroidism on the expression of bsep and mdr2 mRNA in rat liver. A: typical results of agarose gel electrophoresis of the PCR products from liver of hyperthyroid rats (T_4) or euthyroid rats (control). B: densitometric quantification of bsep and mdr2 mRNA. Each column represents the mean \pm SE of 3 rats.

FIG. 4. Effect of hyperthyroidism on the expression of mdr1a (A) and mdr1b (B) mRNA in rat jejunum and ileum. Upper panel: typical results of agarose gel electrophoresis of the PCR products from each tissue of hyperthyroid rats (T_4) or euthyroid rats (control). Six points were selected with serial dilutions of mdr1a/1b mimic competitor cDNA from 0.25 to 50 amol. Lower panel: densitometric quantification of mdr1a and mdr1b mRNA. Each column represents the mean \pm SE of 4 rats. * $p < 0.05$, significantly different from control.

Fig. 1.

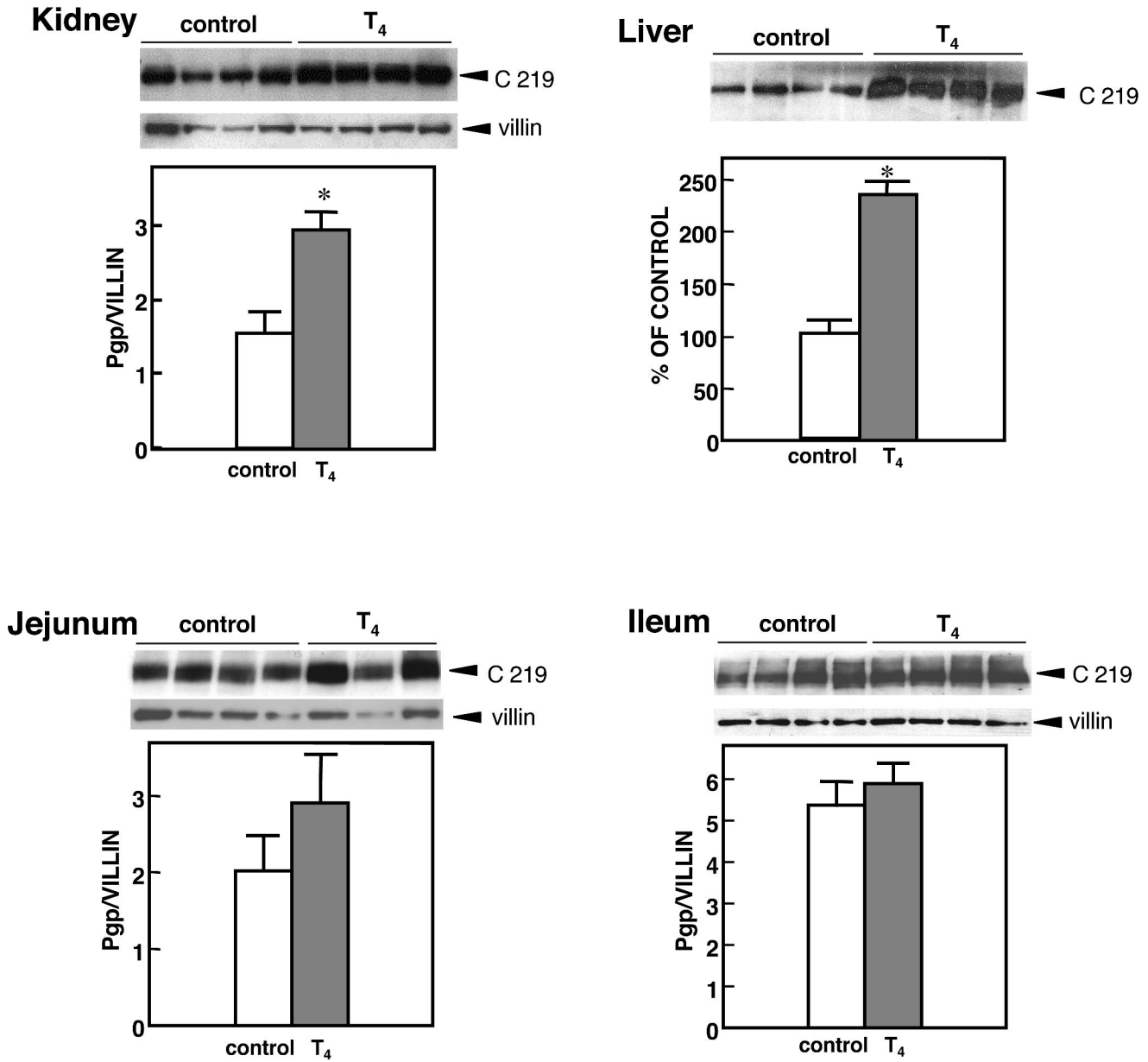


Fig. 2

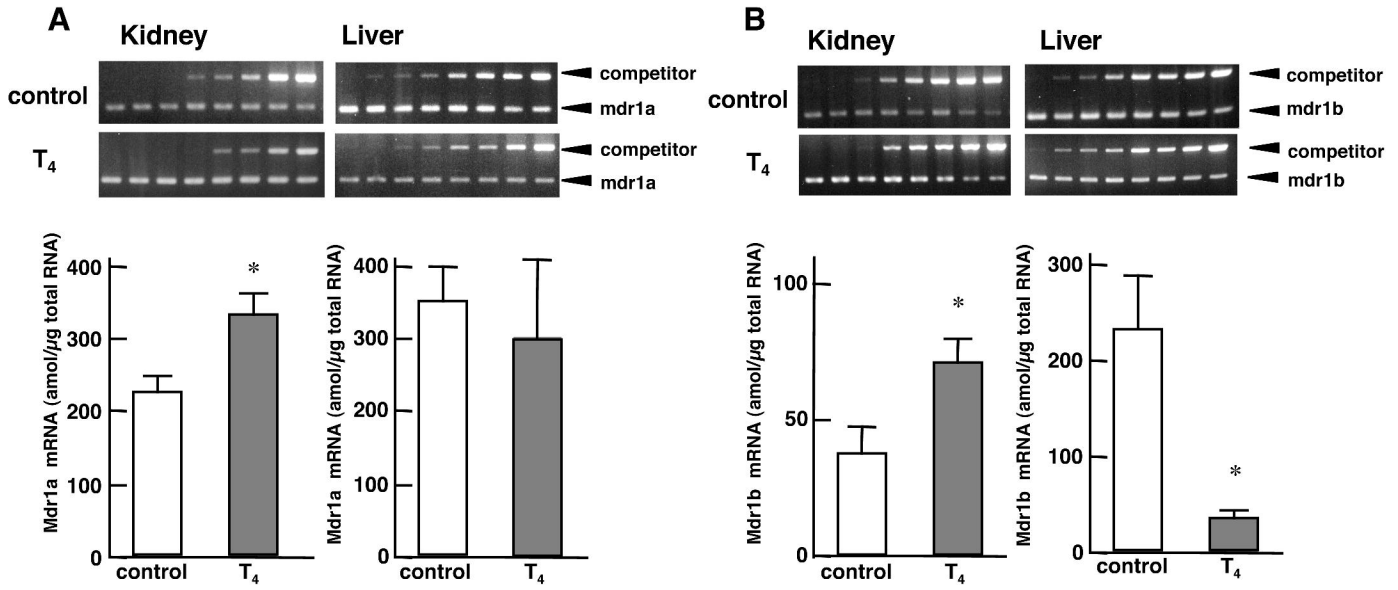


Fig. 3

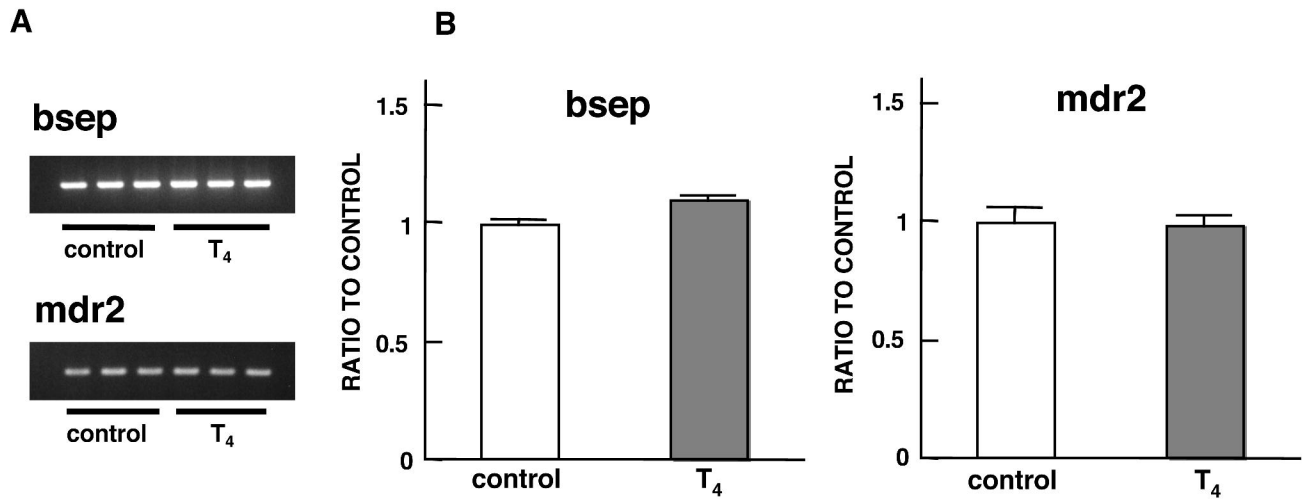


Fig. 4

