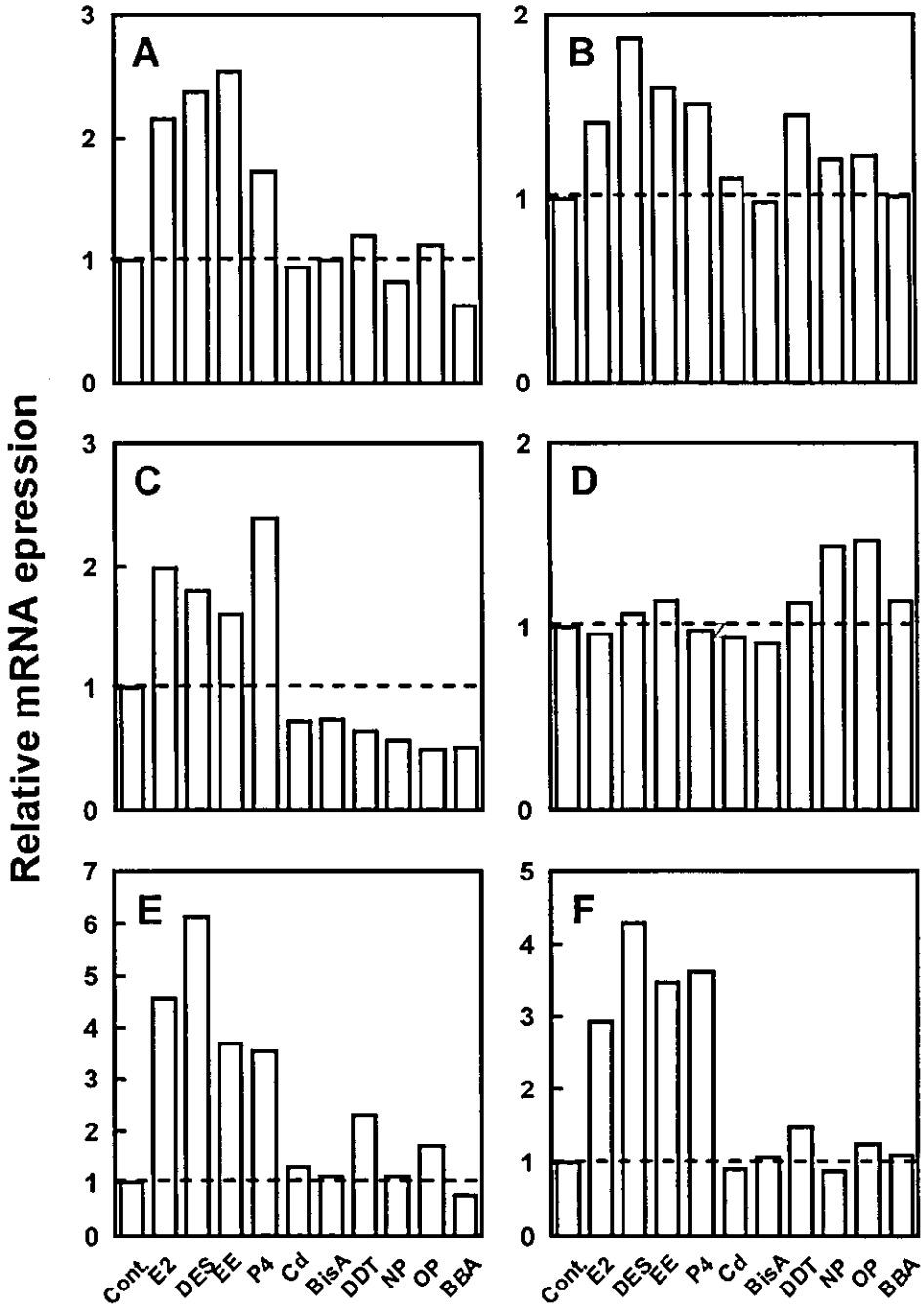

Fig. 1. Effects of various chemical compounds on mRNA expression of P450scc (A), 3 β-HSD I (B), stearyl sulfatase (C), 17β-HSD I (D), aromatase (E), hCG (F) in JEG-3 cells. Total RNA were isolated from JEG-3 cells treated with E2, DES, EE, P4, NP (3×10^{-6} M), Cd (10^{-6} M), Bis A, DDT OP, and BBP (10^{-5} M) for 48 h.

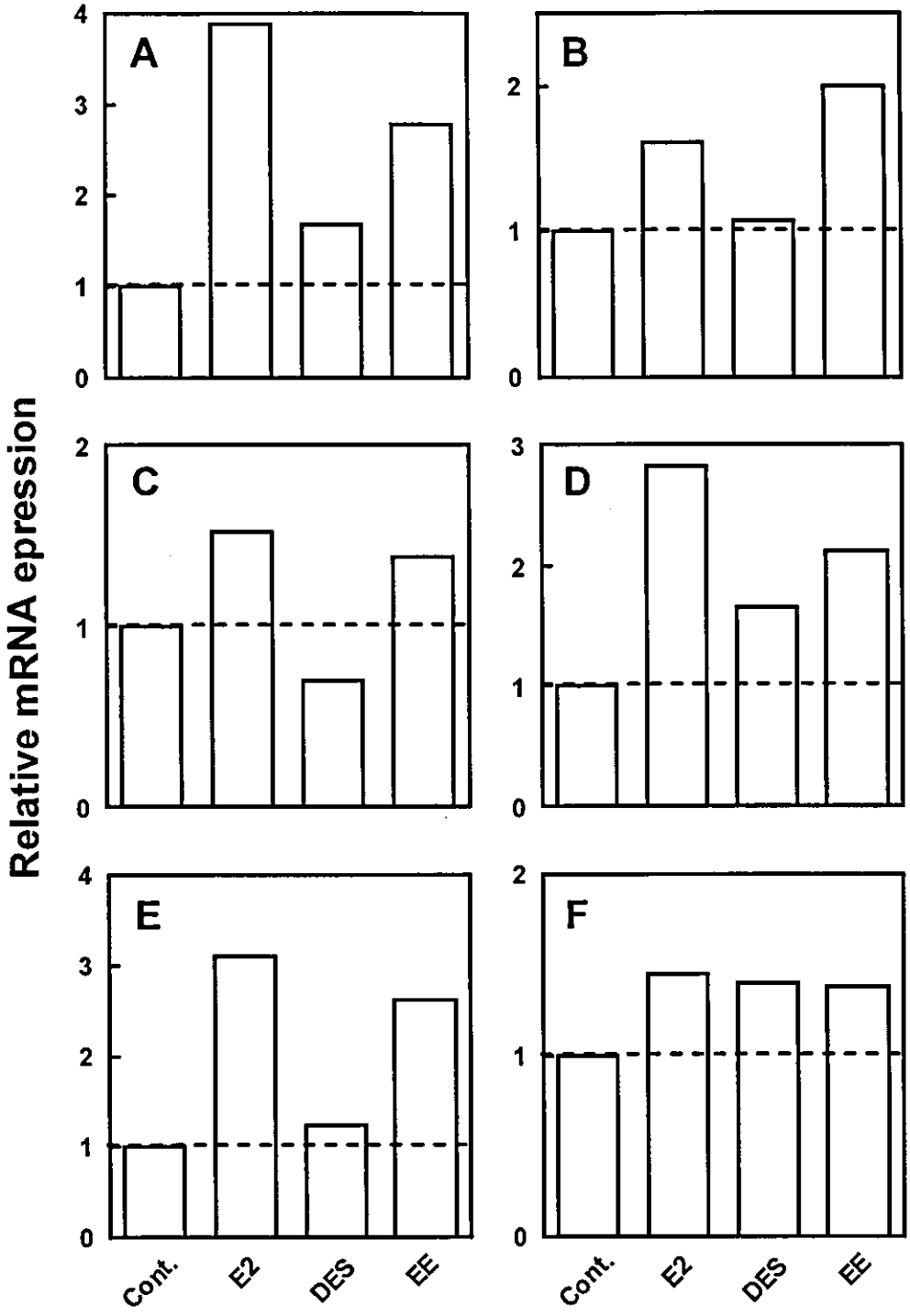

Fig. 2. Effects of various chemical compounds on mRNA expression of P450scc (A), 3 β-HSD I (B), CYP17 (C), 17β-HSD II (D), PL-II (E), PLP-A (F) in Rcho-1 cells. Total RNA were isolated from Rcho-1 cells treated with 3 x 10^{-6} M of E2, DES and EE for 48 h,

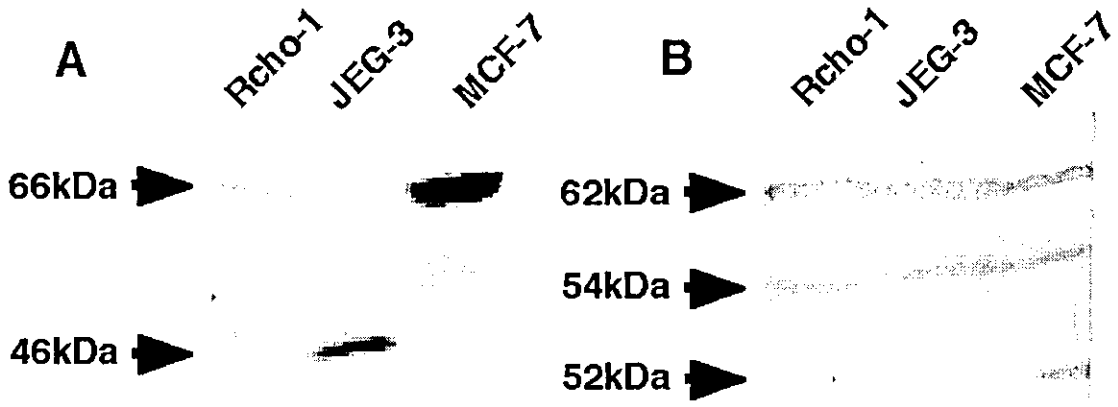

Fig. 3. Western blot analysis of ERs in MCF-7, JEG-3, and Rcho-1 cells. 50 μg of each cell lysate were dissolved on a 10% SDS-poly acrylamide gel and then subjected to immunoblotting with rabbit anti ERα (A) or rabbit anti ERβ (B) antibody.

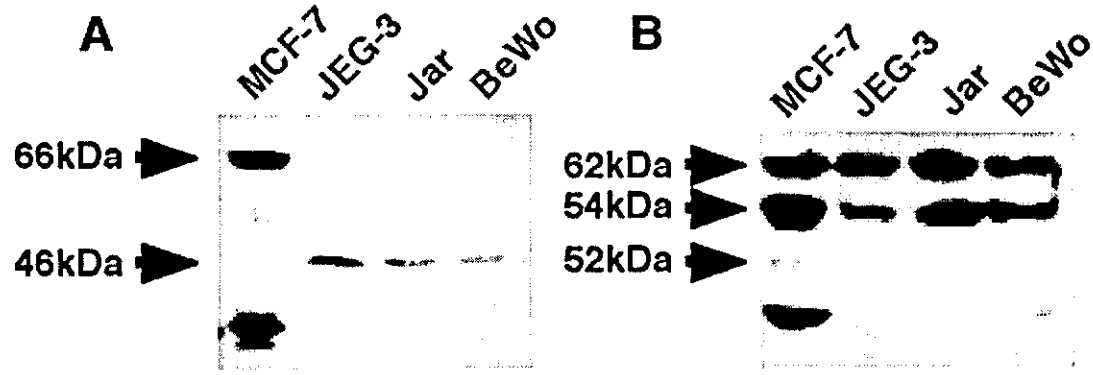

Fig. 4. Western blot analysis of ERs in MCF-7, JEG-3, Jar and BeWo cells. 50 μg of each cell lysate were dissolved on a 10% SDS-poly acrylamide gel and then subjected to immunoblotting with rabbit anti ERα (A) or rabbit anti ERβ (B) antibody.

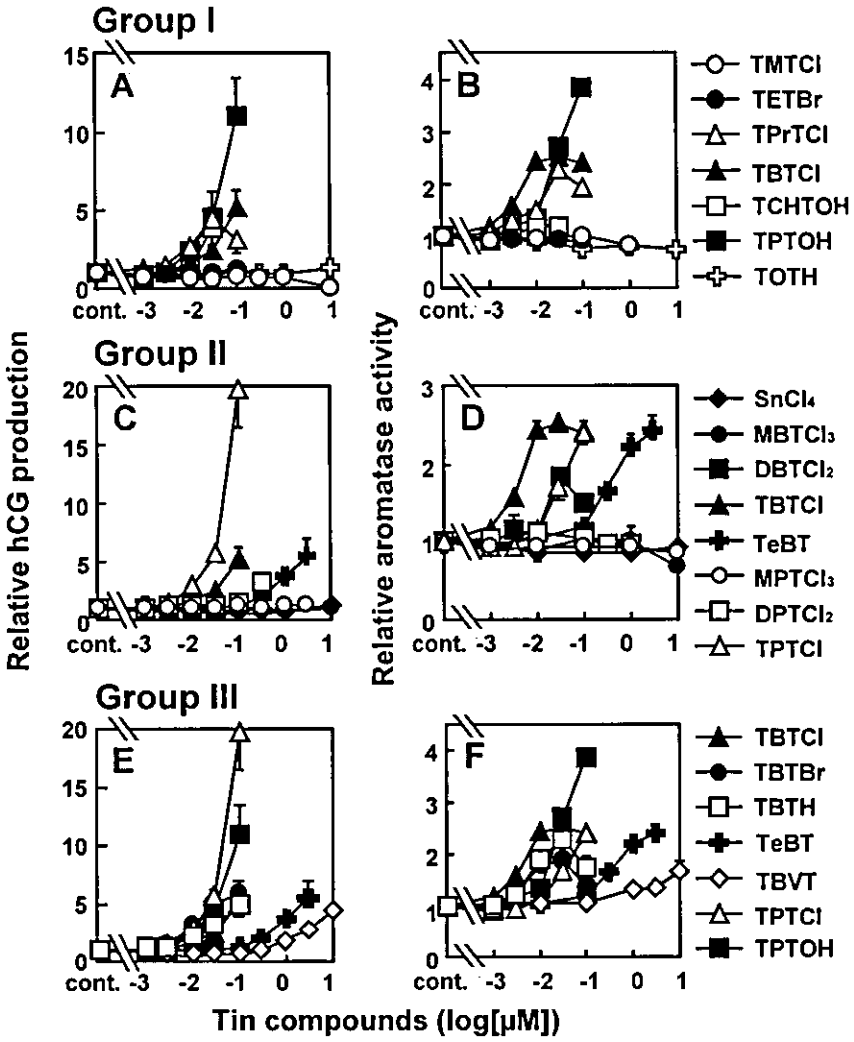

Fig. 5. Effects of tin compounds on hCG secretion (A, C, E) and aromatase activity (B, D, F) in Jar cells. Cells were treated with various nontoxic concentrations of tin compounds for 48 h. A nontoxic concentration of a tin compound was defined as a concentration at which the uptake of [^{3}H]thymidine was ≥ 80% that for the vehicle alone (data not shown). Results are expressed as mean ± 1 SD of triplicate cultures. Group I (A, B): Comparison of different lengths of alkyl chains in trialkyltin compounds. Group II (C, D): Comparison of different numbers of alkyl chains in butyltin and phenyltin compounds. Group III (E, F): Comparison of different fourth function groups on the tin of tributyltin and triphenyltin. The hCG production and aromatase activity in vehicle-only cells, calculated from all experiments, were 290.0 ± 85.3 mIU/well/24 h and 4.08 ± 0.91 pmol/well/ 4 h, respectively.

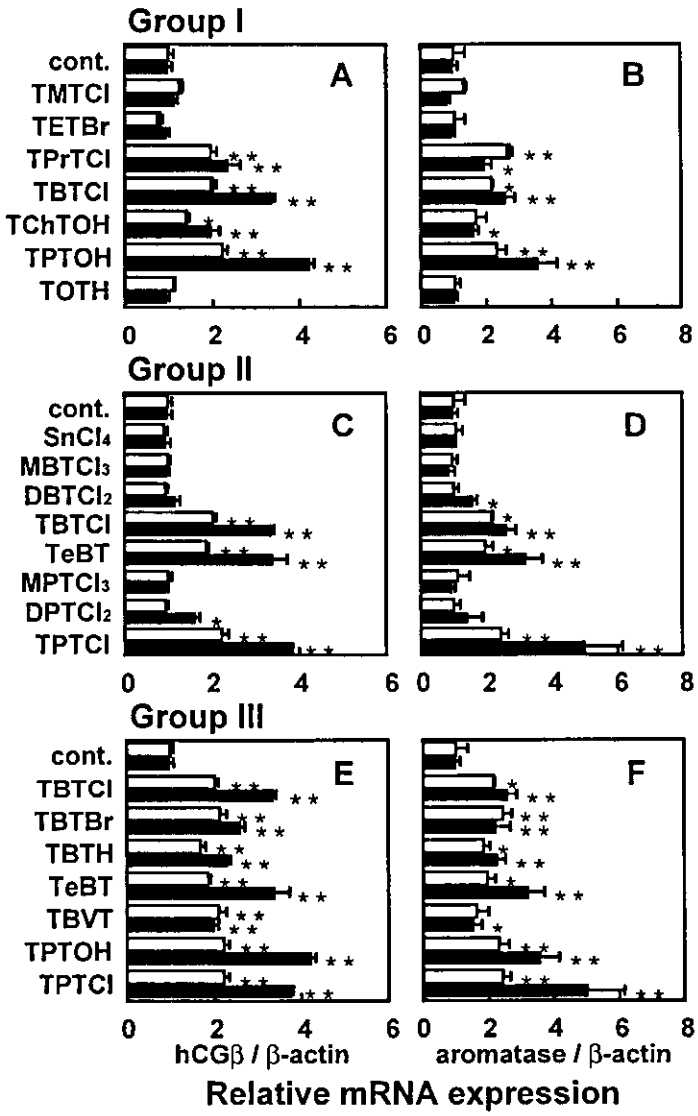

Fig. 6. Effects of tin compounds on the mRNA expression of hCG β (A, C, E) and aromatase (B, D, F) in Jar cells. Total RNA isolated from Jar cells was treated with tin compounds for 24 h (open bars) and 48 h (closed bars). The doses of each compound were: 10 μM of TOTH, $SnCl_4$, $MBTCl_3$ and TBVT; 3 μM of $MPTCl_3$ and TeBT; 1 μM of 9cRA and TMTCl; 300 nM of $DPTCl_2$; 100 nM of TETBr, TBTCl, TPTOH, TPTCl, TBTBr and TBTH; 30 nM of TPrTCl, TChTOH and $DBTCl_2$. The relative hCGβ and aromatase mRNA levels for each condition were determined by quantitative RT-PCR 3 times for each of the 3 independent cultures. Results are expressed as mean ± 1 SD of 3 independent cultures. Groups I (A, B), II (C, D), and III (E, F) correspond to the groups described in the legend for Fig. 5. *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.005$ represent values significantly different controls.

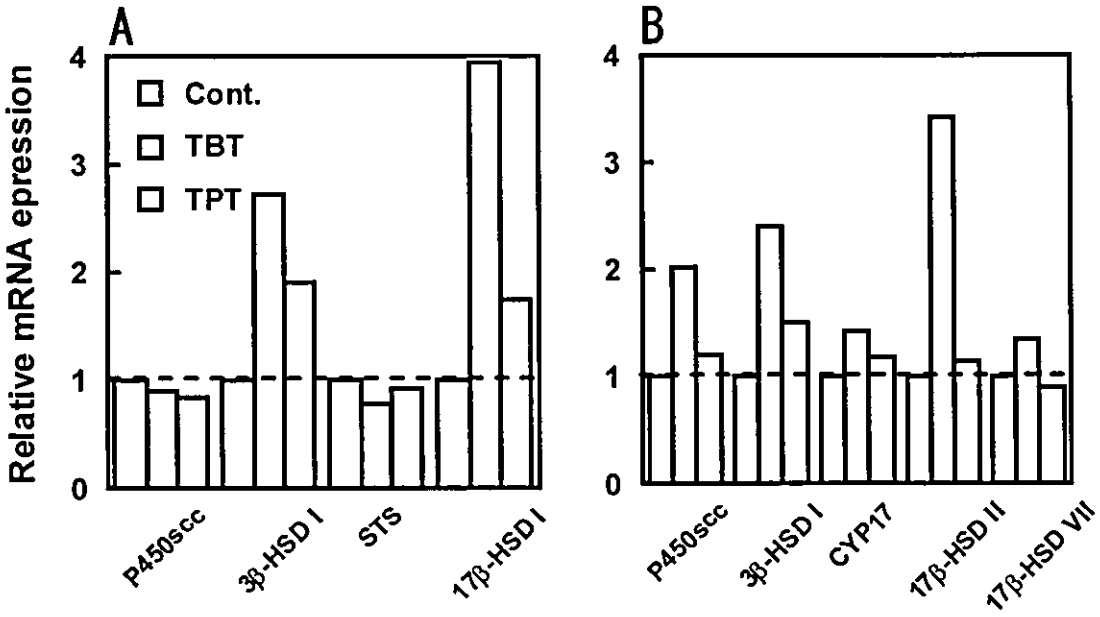

Fig. 7. Effects of organotin compounds on mRNA expression of various enzymes in JEG-3 (A) and Rcho-1 (B) cells. Total RNA were isolated from cells treated with 10^{-7} M of TBT or 3 x 10^{-8} M of TPT for 48 h.

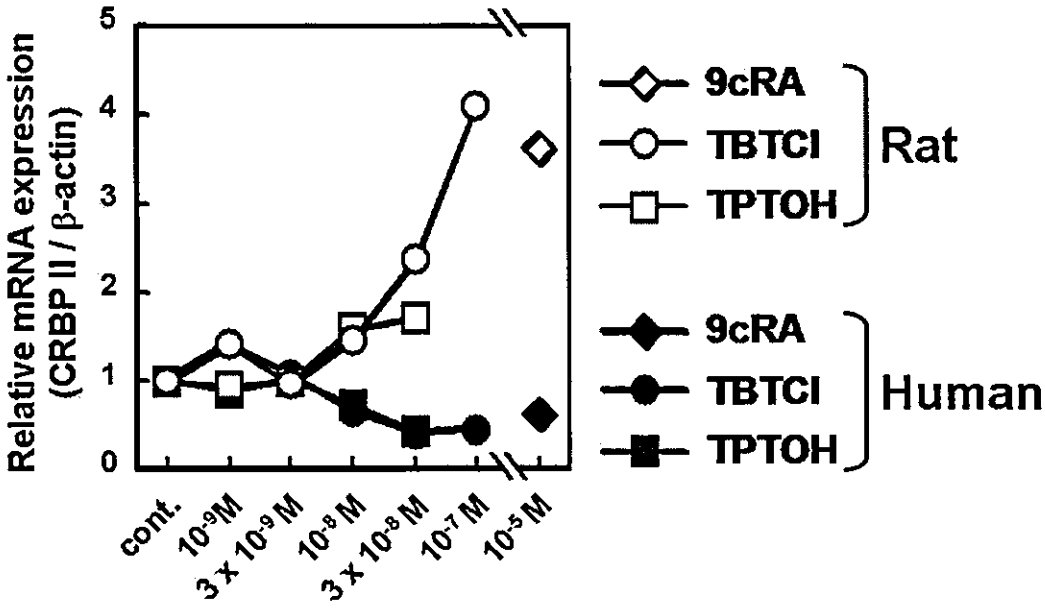

Fig. 8. Effects of organotin compounds on mRNA expression of cellular retinol binding protein (CRBP) II in JEG-3 (human) and Rcho-1 (Rat) cells. Total RNA were isolated from cells treated with TBTCl or TPTOH for 48 h.

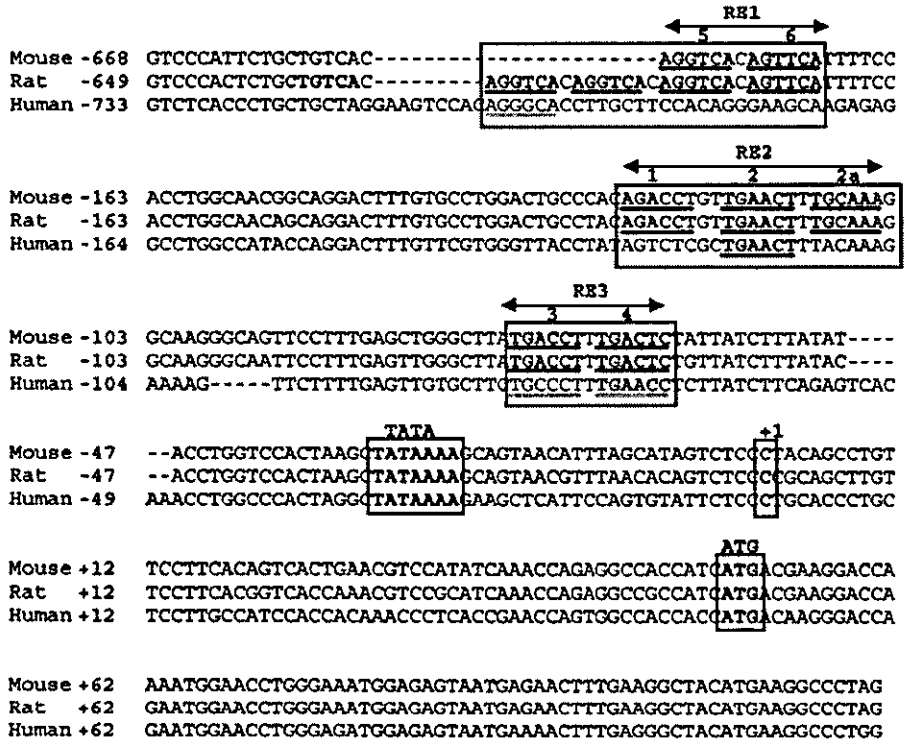

Fig. 8. Genomic organization of the 5' -end of the CRBP II gene as determined by restriction mapping and sequencing. Alignment of the 5'-flanking sequences of human, mouse, and rat *CRBP II* genes. The repeated sequence motifs 5'-RG(G/T)TCA or related motifs [5'-TGA(A/C)CY on the other strand] are in bold. Arrows mark *cis*-acting response elements labeled RE1, RE2, and RE3 in the mouse gene, and the repeated sequence motifs are numbered 1-6. In the rat gene, there are 5 tandemly repeated motifs, which represent the rat CRBP II retinoid x receptor (RXR) response element (RXRE). No region of repeated sequence motifs homologous to the rat CRBP II RXRE and the mouse CRBP II RE1 are found within this 2.8-kb region of the human CRBP II promoter. The TATA box and the initiation codon (ATG) are also in bold.

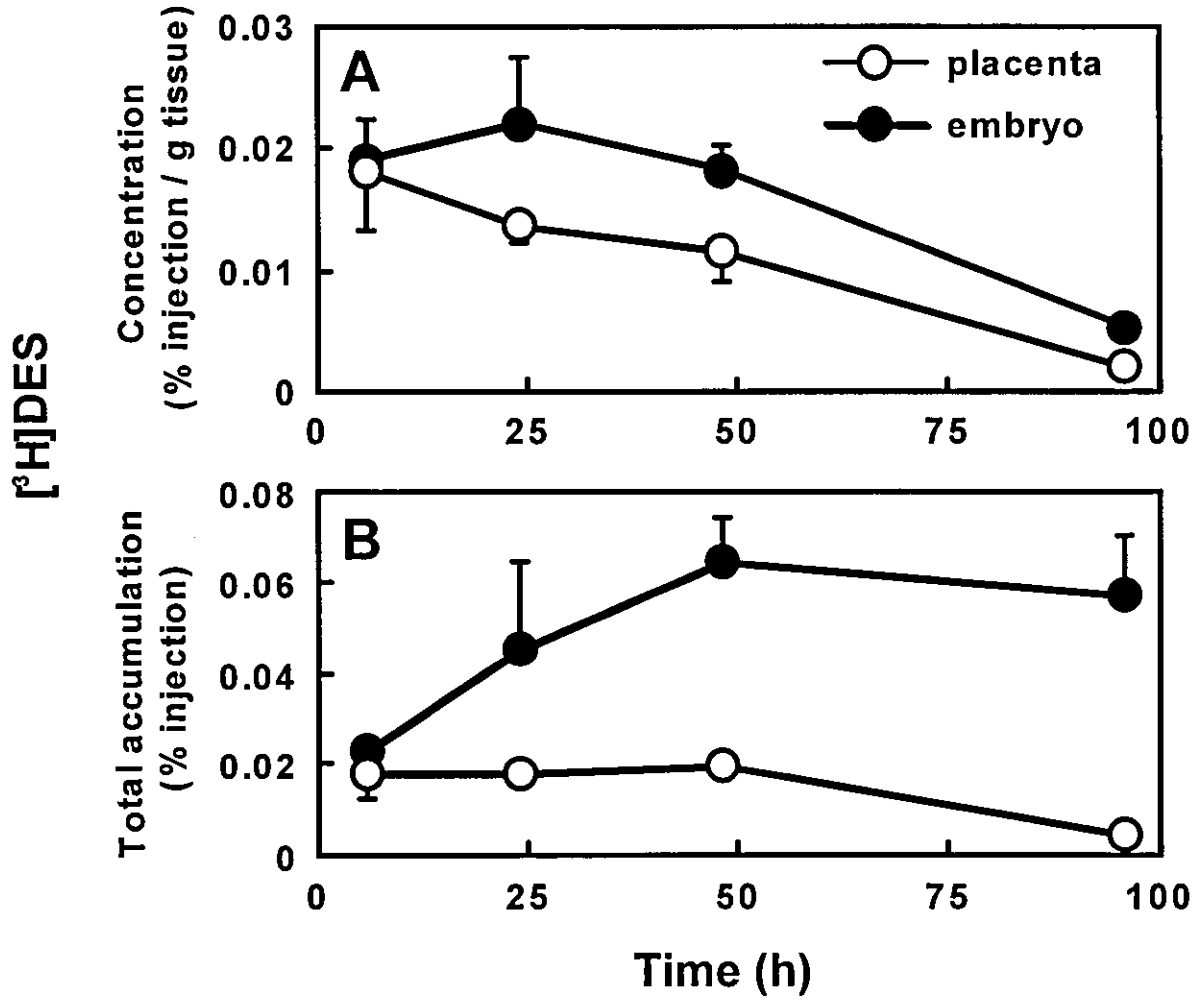

Fig. 10. Time course for total amount of [³H]DES in placenta and embryo of ICR pregnant mice after i.p injection.

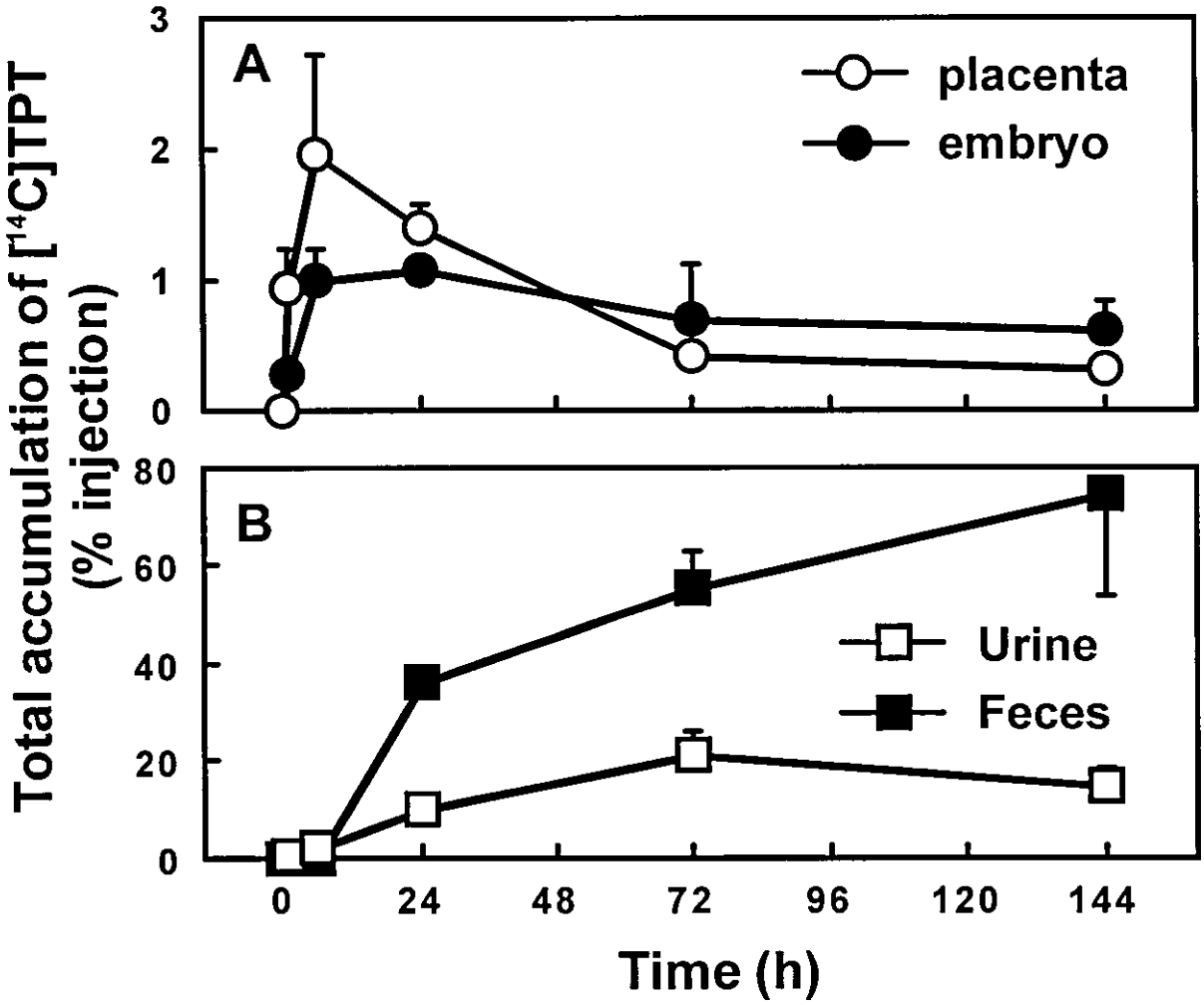

Fig. 11. Time course for total amount of [^{14}C]-TPT OHin placenta, embryo, fuces and urines of ICR pregnant mice after i.p injection.

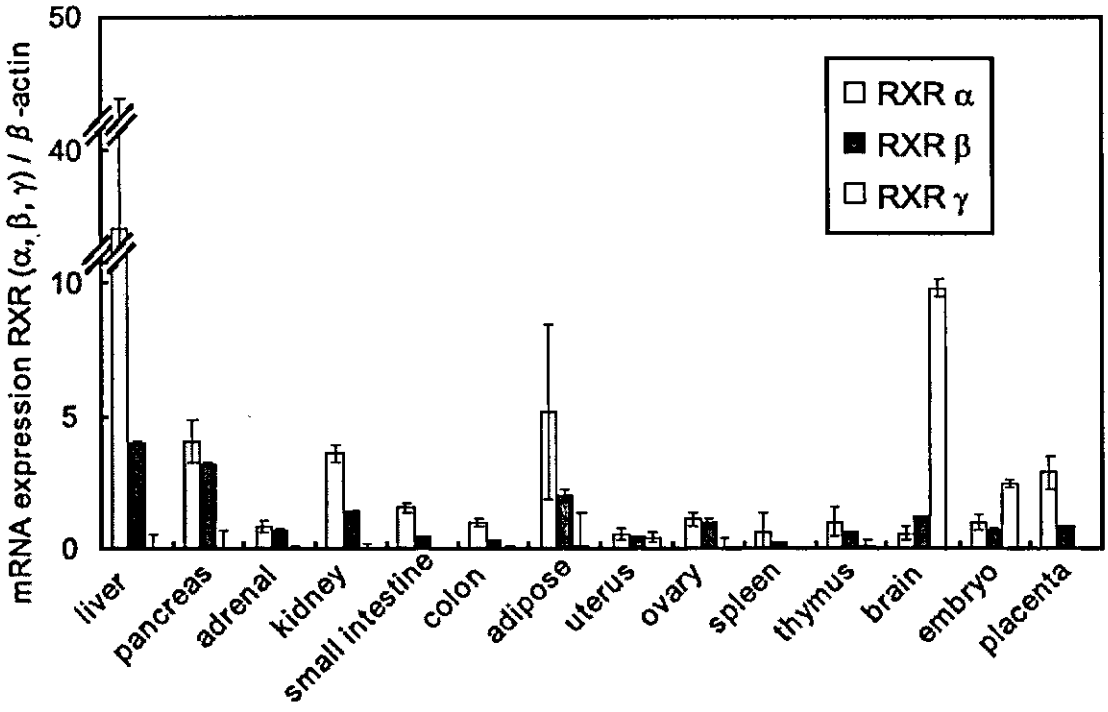

Fig. 12. Transcription level of RXRα, β and γ in tissues of pregnant mice. Total RNA were isolated from tissues of pregnant mouse. RXRα, β, γ, and β-actin expression was analyzed by quantitative real time PCR. Data represent means$\pm$S.D. of triplicate samples

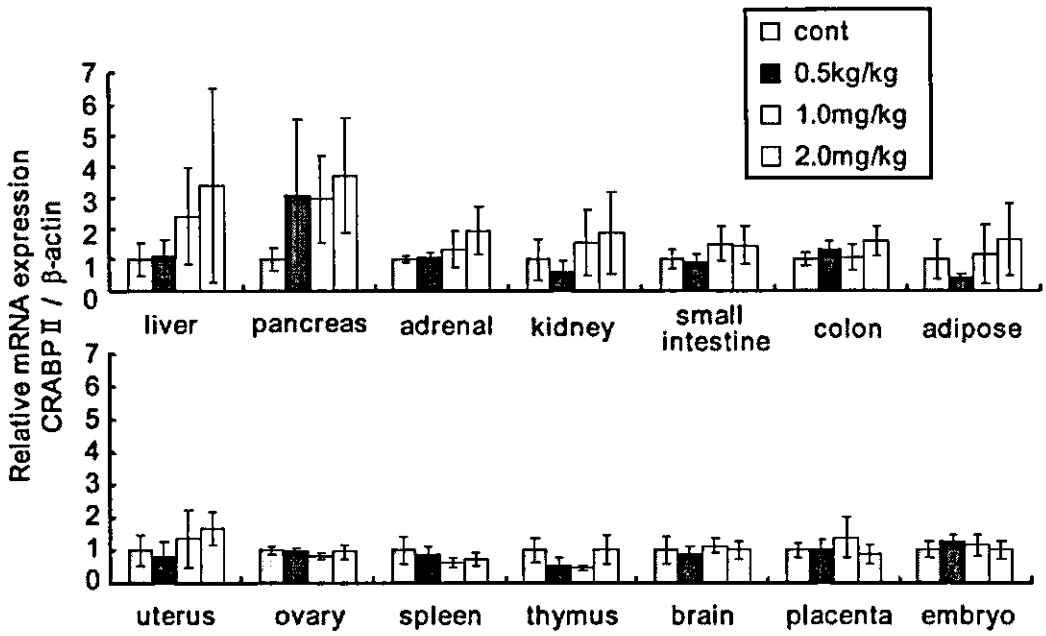

Fig. 13. Effect of TPTOH on CRABPII mRNA expression in the various tissues of pregnant mice. Pregnant mice (n=4) were administrated TPTOH (0, 0.5, 1.0, 2.0mg/kg i.p.). After 72h, each tissue was removed and total RNA was isolated. CRABPII gene expression was analyzed by quantitative real time PCR. Data represent means±S.D. of quadruplicate samples.

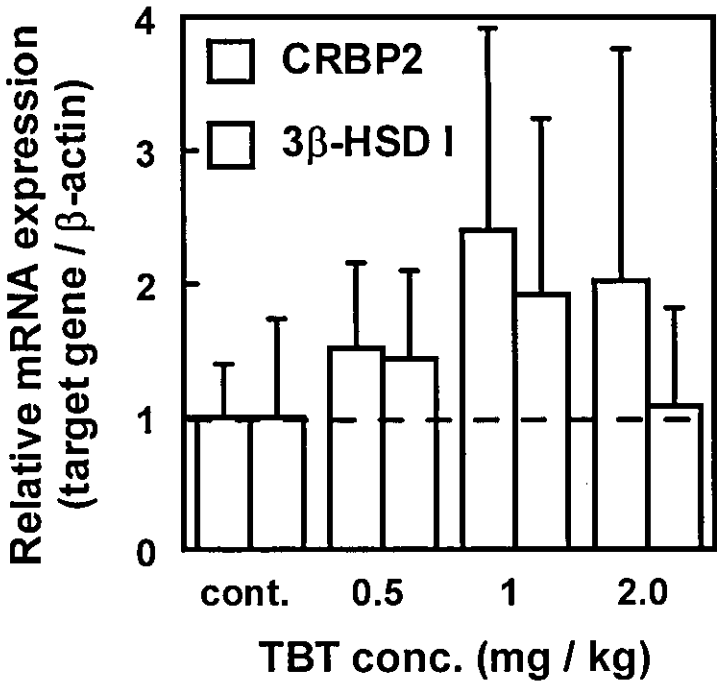

Fig. 14. Effect of TBTCl on mRNA expression of CRBPII and 3β-HSD I in the placenta of pregnant mice. Pregnant mice (n=4) were administrated TBTCl (0, 0.5, 1.0, 2.0mg/kg i.p.). After 72h, the placenta was removed and total RNA was isolated. Each gene expression was analyzed by quantitative real time RT-PCR. Data represent means±S.D. of quadruplicate samples.

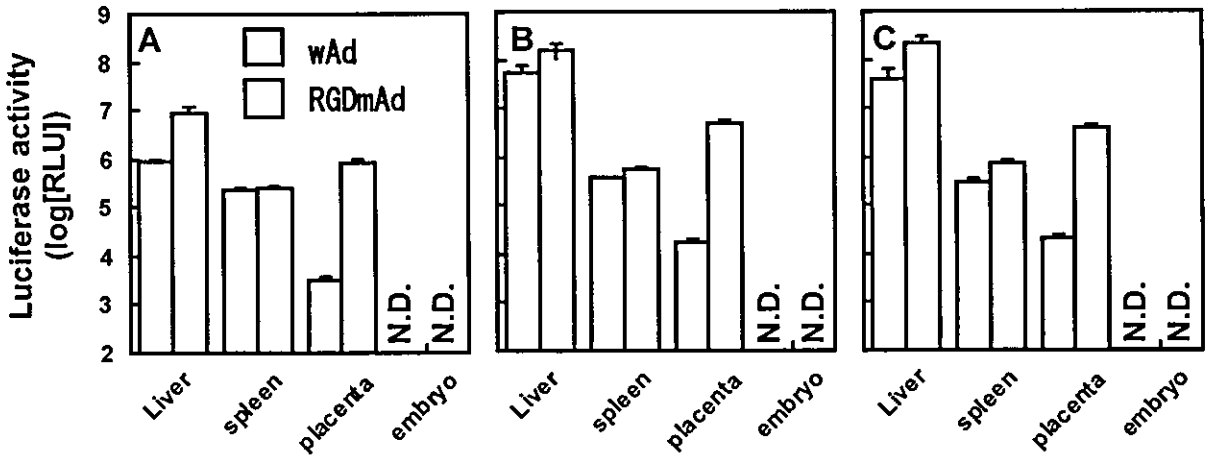

Fig. 15. Transgene expression in ddY pregnant mice after i.v. injection of wAd-CMV-Luc and RGDmAd-CMV-Luc on day 8 (A), 13(B) and 15(C) of pregnancy.

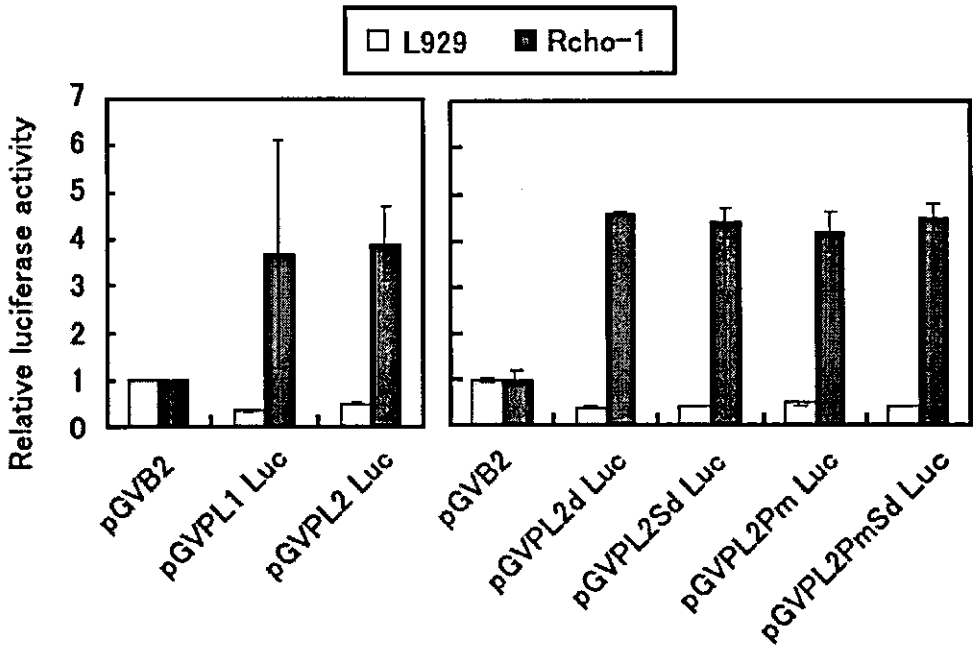

Fig. 16. Trophoblast-specific activity of PL I and PL II promoter in Rcho-1 cells and L929 cells. LUC reporter gene activities under the PL I or PL II promoter regions were measured from extracts of Rcho-1 and L929 cells transiently transfected by PL I or PL II promoter construct. Data represent means ± S.D. of triplicate cultures.

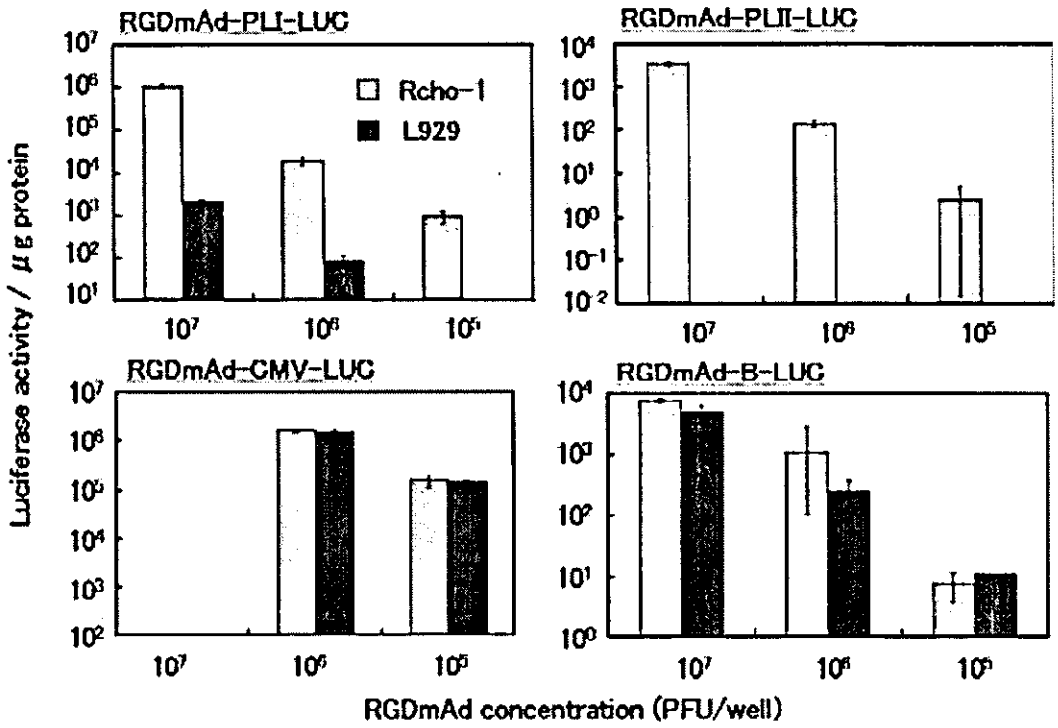

Fig. 17. Transgene expression in Rcho-1 and L929 cells after infection of RGD mAd. Rcho-1 and L929 cells were infected by each RGDmAd (10^5, 10^6, 10^7 PFU / well). After 48h, LUC reporter gene activities were measured from extracts of Rcho-1 and L929 cells infected. Data represent means±S.D. of triplicate cultures.

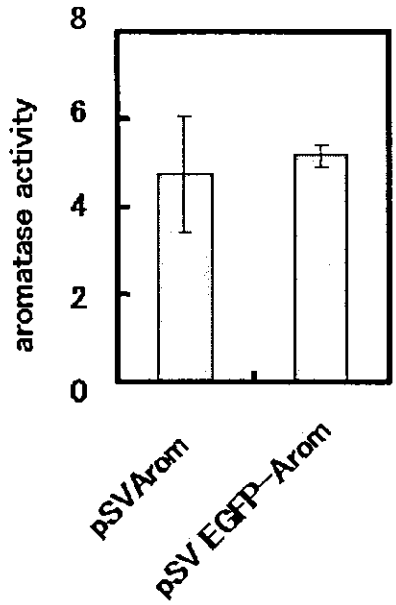

Fig. 18. Aromatase activity in L929 cells transfected by constructs. Aromatase activities were measured from extracts of L929 cells transiently transfected by each construct. Data represent means±S.D. of triplicate samples.

別添４

研究成果の刊行物に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
特になし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakanishi T, Itoh N, Tanaka K	Effects of organotin compounds on endocrine functions of human placental cells.	Toxicol. Appl. Pharmacol.	197	231-232	2004
Nakanishi T, Kohroki J, Suzuki S, Ishizaki J, Hiromori Y, Takasuga S, Itoh N, Watanabe Y, Utoguchi N, Tanaka K	Trialkyltin compounds enhance human CG secretion and aromatase activity in human placental choriocarcinoma cells.	J. Clin. Endocinol. Metab.	87	2830-2837	2002

The estrogen receptor (ER) is a nuclear protein that contains a hormone- and a DNA-binding domain and exerts its effects by binding to *cis*-acting element in the regulatory region of target genes. Therefore, estrogen or estrogen mimics (estrogenic compounds) is retained in target cells by affinity binding to specific estrogen receptor.

Our previous results demonstrated that the expression of medaka choriogenin was activated by estrogenic chemicals, while it was inhibited by anti-estrogenic compounds. At present, In order to characterize the regulatory elements of the choriogenin genes, the upstream region of the genes was cloned and sequenced. ERE (Estrogen Responsive Elements) sequences were identified in the regulatory region of the choriogenin L and choriogenin H genes. They are located between −340 and −200 from the transcription initiation site. To assess *cis*-binding requirements to ER to its epecific DNA elements of the choriogenin, ER binding to the ERE region of the medaka choriogenin was examined by using gel mobility shift assay. The levels of DNA-protein complexes were significantly increased in the reaction mixture composed of labeled oligonucleotides containing EREs and the liver extracts from the estrogen-treated medaka males. Also the binding complex of purified ER-alpha and choriogenin ERE could be formed. From the above results, choriogenin mRNA expression proved to be induced and promoted by forming the ER-ERE complex. Also these results indicate that the medaka choriogenin gene is ertrogen-regulated directly and can be a suitable biomarker for environmental estrogens.

406

APPLICABILITY OF IN VITRO ASSAYS FOR SCREENING OF ENDOCRINE DISRUPTORS: RELATIONSHIPS BETWEEN IN VITRO AND IN VIVO SCREENING ASSAYS

M. Nakai, M. Takeyoshi, S. Noda, Y. Akahori, Y. Minobe, Y. Yakabe, and K. Yamasaki, *Chemicals Evaluation and Research Institute, Japan (CERI)*

In these years, several in vitro and in vivo assays for screening of endocrine disruptors have been developed. To screen the mechanism-specific endocrine disrupting potencies of numerous chemicals in a short term at a low price, it is indispensable to establish the reliable in vitro assays predicting the biological effects of chemicals with high accuracy. However, there is little research that clearly demonstrates the concordance of potencies between in vitro and in vivo screening methods based on receptor-mediated mechanism. Bindings of chemicals to estrogen receptor (ER) followed by gene transcriptional regulation are known as one of the key modes of actions that may disrupt the endocrine system. Therefore, in this study, we focused on the ER-mediated endocrine disrupting mechanism of chemicals. In order to evaluate the applicability of in vitro assays for screening of endocrine disruptors, ER-binding and ER-mediated transcriptional assays were compared to rat uterotrophic assay on 38 chemicals, respectively. Since rat uterine weight is increased depending on estrogenic activity of chemical exposed, uterotrophic assay is a typical in vivo screening assay detecting ER-mediated effect of chemical. Relative binding affinity (RBA), which was a percent ratio of IC50 values of E2 and chemicals, was employed for comparison of the receptor-binding potency among chemicals. Transcriptional activity of chemical was designated by a 10 percent-potent concentration (PC10), which defined as a concentration of test chemical activating at 10% of maximal transcriptional activity by E2. The lowest effective dose (LED) was utilized for quantitative analysis of estrogenic activity of chemical in a three-day uterotrophic assay on immature rat. RBA values showed good correlation with LED values and the concordance between ER-binding and uterotrophic assays was 87%. PC10 also exhibited excellent relationship with uterotrophic assay and the concordance between them was 89%. These results suggested that in vitro ER-binding and ER-mediated transcriptional assays were applicable to screen the endocrine disruptors, which act mediated by ER-binding.

407

DI(2-ETHYLHEXYL)PHTHALATE MAY REDUCE MOUSE FERTILITY VIA PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α (PPARα)

T. Nakajima,[1] Y. Hata,[2] Y. Ito,[1] M. Omura,[3] H. Sone,[4] C. Toyama,[4] F.J. Gonzalez,[5] and T. Aoyama[2], [1]*Nagoya University Graduate School of Medicine, Nagoya, Japan*, [2]*Shinshu University School of Medicine*, [3]*Kyushu University Graduate School of Medical Sciences*, [4]*National Institute for Environmental Studies*, [5]*National Cancer Institutes, USA*

Di(2-ethylhexyl)phthalate (DEHP)-induced reduction of fertility and its role of PPARá, were assessed using wild-type and PPARá-null mice. All mice were given 0 or 0.05% DEHP-containing diets ad libitum throughout the experiment. Male and female pups (F_0) on the same dose and of the same genetic strain were mated after dosing at 0 or 0.05% DEHP for 4 weeks, and their pups (F_1) were further mated at maturity in the same manner as the F_0 generation. The resulting pups were designated as the F_2 generation. DEHP treatment induced the expression of hepatic peroxisomal enzymes in both mature males and females of the wild type, but not in their fetuses and pups, suggesting that the dose activates PPARá function only in mice fed DEHP-containing diets. No morphological abnormality was found in the genital organs from mature males and females of both genotypes under microscopic observations. Nevertheless, in the wild-type mice, DEHP exposure caused a reduction in the numbers of pups born and those that survived for 16 weeks per pair in both F1 and F2 generations. In another experiment involving 0, 0.01 and 0.05% DEHP exposure of both genotype mice, it was found that a reduction in the numbers of pups born was due to an increase in the resorption of fetuses; a reduction in those of surviving pups resulted from an increase in the mortality rates of newborn pups. Exposure to 0.05% DEHP also decreased body weight of pups only in wild-type mice. In contrast, PPARá-null mice were refractory to the adverse effects of DEHP observed in the wild-type mice in all generations. These results suggest that the reduction of fertility without the maternal or paternal genital organ toxicity found in DEHP-exposed wild-type mice is partly caused by a signal triggering energy overconsumption via PPARá in the maternal body.

408

EFFECTS OF ORGANOTIN COMPOUNDS ON ENDOCRINE FUNCTIONS OF HUMAN PLACENTAL CELLS

Tsuyoshi Nakanishi, Norio Itoh, and Keiichi Tanaka, *Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan*

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), are known to induce an irreversible sexual abnormality of female neogastropod snails, which is termed "imposex". Human exposure to these organotin compounds may result from the consumption of organotin-contaminated meat and fish products. However, in humans, it remains unclear whether organotin compounds disturb the sexual development and reproduction. The principal hormones, estrogens and human chorionic gonadotropin (hCG) are produced by the placenta during pregnancy. These hormones are essential to several important events in establishment and maintenance of preg-