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REGULATION OF SELENOPROTEIN P mRNA EXPRESSION IN COMPARISON WITH METALLOTHIONEIN AND OSTEONECTIN mRNAs FOLLOWING CADMIUM AND DEXAMETHASONE ADMINISTRATION

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INDEXING WORDS

selenoprotein P; metallothionein; osteonectin; cadmium; dexamethasone

SYNOPSIS

Selenium is recognized as an essential trace element and an antidote for carcinogens, heavy metals etc., and also as an environmental pollutant causing dysfunction of both the brain and peripheral tissues. Selenoprotein P (SelP) contains 10 selenium per molecule in the form of selenocysteines. To clarify whether SelP is involved in selenium requirement and toxicity, SelP mRNA expression was compared with the expression of metallothionein (MT) and osteonectin (OST) mRNAs, the protein products of which are known to have antidote effects. MT and OST are induced by diverse forms of stress and immediately affect genes with stress promoter sequences. Cd and dexamethasone were used to examine such secondary regulation. Basal expression of SelP mRNA was high both in NRK cells and in rat kidney and brain but dexamethasone induction was observed only in NRK cells. Dexamethasone, but not Cd, decreased expression of OST mRNA in NRK cells, while OST mRNA in the kidney and brain increased after Cd administration in rats. Induction of MT mRNA was observed in response to Cd and dexamethasone in all cells and tissues examined, while the net increase was little because its basal expression was faint. Moreover, in situ hybridization indicated that SelP mRNA expression was localized to the cerebellum, one of the targets of selenium toxicity. The cerebellum is also a target for methyl-Hg intoxication, symptoms of which are ameliorated by selenium. Thus, SelP seems to be involved in both selenium homeostasis and detoxication mechanisms even though SelP mRNA is not always inducible.

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INTRODUCTION

Selenium has been recognized as an essential trace element because its deficiency causes a cardiomyopathy affecting infants and women in China known as Keshan disease.[5,6] In addition to a protective effect against carcinogenic agents, selenium compounds display an antidote effect against heavy metal intoxication caused by methyl-Hg, Cd etc.[6] However, excess selenium leads to "blind stagger", a neuronal syndrome including visual disturbances, gait stagger, abdominal pain etc. as well as "alkali disease" including muscular disorders, cardiac atrophy, liver cirrhosis, etc.[6] Thus, it seems important to clarify the disposition of selenium in the body not only in selenium deficiency but also in selenium intoxication.

Cellular glutathione peroxidase, which scavenges hydroperoxides, and iodothyronine 5'-type-I-deiodinase, which transforms thyroid hormone T3 into the active T4, contain only one selenium per subunit in the form of selenocysteine at their catalytic domain and it is unlikely that these enzymes play roles in the maintenance of selenium homeostasis.[1,16] On the other hand, selenoprotein P (SelP)[8] and SelP-like protein[14] contain 10 and 12 selenocysteines per molecule, respectively. Their physiological functions beyond their roles as scavengers[3] are not known, and it is not yet clear whether the functions of SelP and SelP-like protein are the same. However, SelP expression was reported to be decreased in selenium deficiency,[2,19] hence it is possible that SelP plays an important role in transposition of selenium. The kidney is the target of diverse forms of stress, while the brain is the target of selenium toxicity in the case of "blind stagger",[6] and selenium improves methyl-Hg accumulation in the brain.[6,10] To confirm that SelP is involved in the antidote effects of some stresses through maintenance of selenium homeostasis, its mRNA expression was examined in NRK cells and in rat kidney and brain following Cd administration. Among the various divalent cations, Cd is the strongest inducer of expression of genes containing the metal responsive element consensus sequence (MRE).[7] As reported previously,[9] such stimuli may alter the levels of expression of metallothionein (MT) and osteonectin (OST) mRNAs. These proteins themselves are known to possess antidote effects and are responsible for transposition of heavy metals,[7] which may cause secondary effects on SelP mRNA expression. Thus, in the present study SelP mRNA expression was compared with expression of MT and OST mRNAs. Since both MT and OST mRNAs are known to be expressed in association with diverse forms of stress,[7,9] it is possible that SelP mRNA expression is regulated secondarily to their induction, which may alter Cd-induced SelP mRNA. Thus, the effects of dexamethasone, one of the strongest inducers of MT mRNA,[7,12,13] on their expression were also examined.

MATERIALS AND METHODS

Cell culture and total RNA preparation

NRK cells (RIKEN Cell Bank, Ibaragi, Japan; RCB0043), derived from normal rat kidney were cultured in MEM with 10% fetal calf serum at 37°C under an atmosphere of 95% air and 5% CO_2. The cells were subcultured every 4 days. When the cultures reached confluency, serum was removed from the media and culture was continued overnight. Thereafter, $CdCl_2$ or dexamethasone was added to the culture media at final concentrations of 10^{-7} - 10^{-6} M or 10^{-8} - 10^{-7} M, respectively, and incubation was continued for 3 or 6 hr. Total RNA was extracted by the method of Davis.[4] Ten μg of total RNA was electrophoresed on 1% agarose gels , transferred to nitrocellulose membranes and fixed with UV light. The filters were hybridized with ^{32}P-random-prime-labeled SelP, MT or OST cDNAs in a buffer containing 40% formamide, 7 mM Tris-HCl (pH 7.4), 1 × Denhardt's solution, 4 × SSC and 10% dextran sulfate at 42°C overnight, washed twice at 65°C in 0.1 × SSC with 0.1% SDS[4] and exposed to X-ray film at -80°C overnight or an imaging plate (Fujix, Tokyo, Japan) at room temperature for 30 min. In order to quantitate hybridization intensities, the plates were analyzed using a Bio Image Analyzer BAS 2000 (Fujix, Tokyo, Japan). The amounts of RNA were calibrated both by the intensity of ethidium bromide staining on a 1% agarose gel and with reference to hybridization of GAPDH as an internal control.

Administration of Cd and dexamethasone to rats and preparation of total RNA and brain slices

Four-week-old male rats (Jcl:Wistar) were subcutaneously injected with saline, $CdCl_2$ (50 μmol/kg) and dexamethasone (1 mmol/kg). Rats were killed 3 or 6 hr after administration, the kidneys and brain were quickly removed and total RNA was extracted for Northern blot analysis as described above. In situ hybridization was performed to investigate in which regions SelP, MT and OST mRNAs were induced by Cd and dexamethasone treatment. For in situ hybridization, the brains were immediately removed and quick-frozen by immersion in liquid nitrogen. Horizontal sections 20 μm thick were cut on a cryostat and thaw-mounted onto poly-L-lysine-coated RNase-free microscope slides. The sections were treated as previously described for hybridization.[17]

In situ hybridization

Hybridization was performed overnight at 50°C with the ^{35}S-random-prime-labeled inserts of SelP, MT and OST in a buffer containing 20 mM Tris-HCl (pH 7.4),

4 × SSC, 50% formamide, 10% dextran sulfate, 1 × Denhardt's solution, 640 μg/ml of salmon sperm DNA, 250 μg/ml of yeast tRNA, 14 mM 2-mercaptoethanol and 10 mM dithiothreitol. After incubation, the slides were washed four times in 0.2 × SSC at 50°C for 15 min each time, then exposed to X-ray film at room temperature for 7 days.[17]

RESULTS

Effects of Cd and dexamethasone on mRNA expression in NRK cells

SelP and OST cDNAs showed intense hybridization to bands of around 2.0 Kb and 3.2 Kb, respectively, in total RNA obtained from NRK cells even in the absence of Cd and dexamethasone. Only faint hybridization to MT cDNA was observed at around 400 b (Fig. 1). Addition of 10^{-7} M Cd to the culture media increased MT mRNA expression within 3 hr. The level of MT mRNA reached more than threefold the control level 6 hr after exposure to 10^{-6} M Cd. Cd administration did not induce a significant increase in expression of either SelP mRNA or OST mRNA. When NRK cells were treated with 10^{-8} M and 10^{-7} M dexamethasone, SelP mRNA was markedly induced and reached approx. 4 times as much as the control level after 6 hr. MT mRNA expression was also induced by 10^{-8} M dexamethasone, whereas OST mRNA expression decreased to 1/2 of the control level. In the NRK cells,

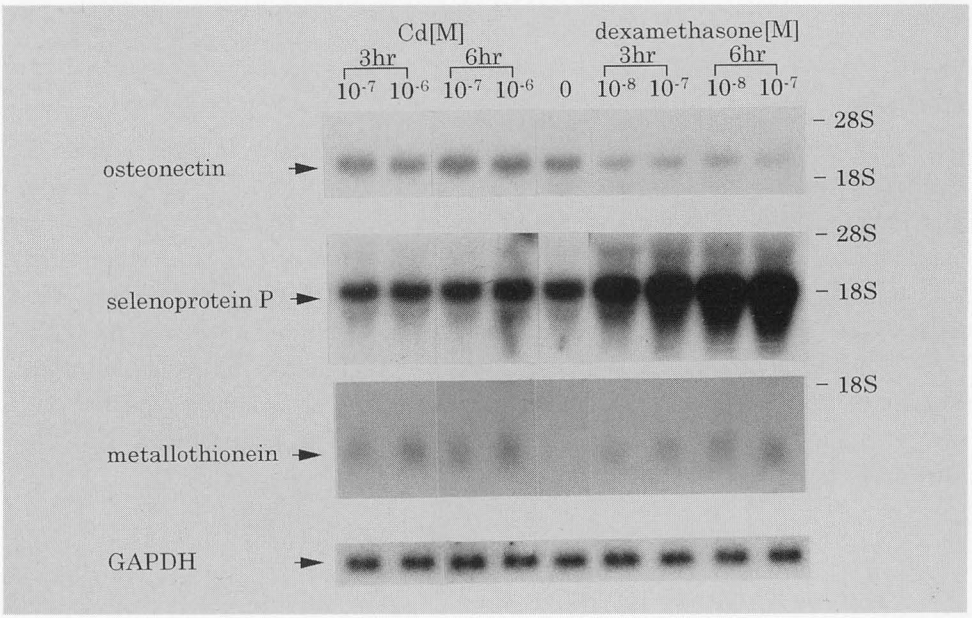

Fig. 1. Northern blot analysis of selenoprotein P, metallothionein, osteonectin and GAPDH mRNAs in NRK cells after treatment with or without Cd and dexamethasone.

SelP mRNA differed from MT and OST mRNAs, in basal expression and responses to Cd and dexamethasone, and hence their patterns of expression were examined in vivo.

Effects of Cd and dexamethasone administration to rats on the kidney and brain mRNAs

The sizes of mRNAs from both rat kidney and brain which hybridized to SelP, MT and OST cDNAs were similar to those from NRK cells (Fig. 2). SelP mRNA was expressed in both the control kidney and brain but the level in the kidney was more than 8 times as much as that in the brain (Fig. 3a). Basal expression of MT mRNA was faint both in the kidney and brain (Fig. 3b). OST mRNA was expressed in the control kidney and brain but was less than 1/3 of the basal level of SelP mRNA expression (Fig.3c). Neither Cd nor dexamethasone induced significant alterations in the SelP mRNA level in the kidney or brain (Fig. 3a). On the other hand, MT mRNA in the kidney increased to approx. 3.7 or 1.8 times of the basal expression 6 hr after administration of Cd or dexamethasone, respectively (Fig.3b). Levels of induction of MT mRNA in the brain by Cd and dexamethasone were less than those in the kidney but were still significant. However, the net increase was little, since the basal expression of MT mRNA itself was extremely low as described above. OST mRNA in the kidney increased to approx. 1.8-fold the basal level after Cd administration but did not change after dexamethasone treatment. In the brain, slight induction of OST mRNA was observed by Cd but no change was observed following dexamethasone treatment (Fig.3c).

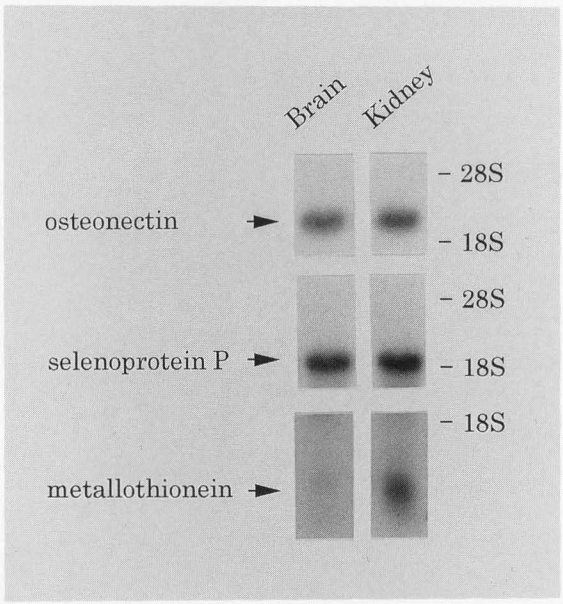

Fig. 2. Northern blot analysis of selenoprotein P, metallothionein and osteonectin mRNAs in the rat kidney and brain.

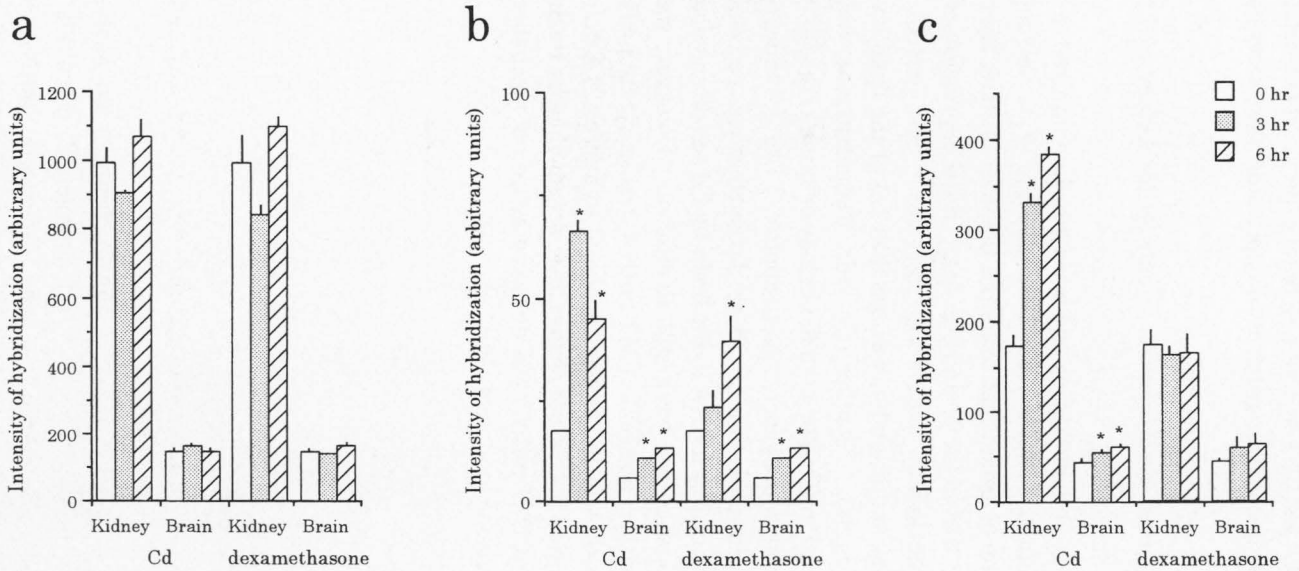

Fig. 3. Effects of Cd or dexamethasone treatment on selenoprotein P (a), metallothionein (b) and osteonectin (c) mRNA expression in rat the kidney and brain. Each bar represents the mean ± S.E. of the intensity of hybridization in the kidneys and brains obtained from four different animals. *Significantly different from the control level ($P<0.05$, one-way ANOVA and post hoc Dunnett's t-test).

In situ hybridization analysis of rat brain

When ^{35}S-labeled inserts of SelP were used as a probe in the control rat brain, prominent hybridization was observed in the pyramidal and granular cell layers of the hippocampal formation and the granular cell layer of the cerebellar cortex (Fig. 4a). Hybridization was also prominent in the granular cell layer and subependymal zone of the olfactory bulb and olfactory tubercle (data not shown). MT mRNA hybridization was observed ubiquitously in the gray matter but not in the white matter (Fig. 4b). Distinct localized hybridization to OST mRNA was obtained in the granular cell layer of the cerebellar cortex, thalamus and striatum (Fig. 4c). However, significant alterations were not observed for any of these three mRNAs expression after Cd or dexamethasone treatment.

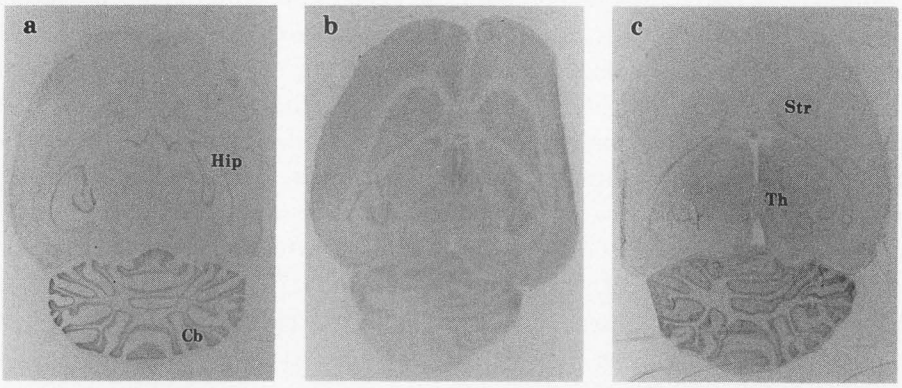

Fig. 4. In situ hybridization of selenoprotein P (a), metallothionein (b) and osteonectin (c) mRNAs in the rat brain.

DISCUSSION

Distinct differences were observed in basal expression pattern and responses to Cd and dexamethasone among SelP, MT and OST mRNAs. SelP and OST mRNAs were constitutively expressed both in NRK cells and in the rat kidney and brain, while only slight MT mRNA expression was observed. MT mRNA was markedly induced by Cd and dexamethasone in NRK cells and all tissues examined. However, responses to dexamethasone of SelP and OST mRNAs in the kidney and brain were different from those observed in the NRK cells.

SelP mRNA was induced in NRK cells by dexamethasone but not by Cd. Although the rat SelP promoter has not yet been cloned, it has been suggested that this gene is positively regulated by the glucocorticoid responsive element consensus

sequence (GRE) but not by MRE. However, no induction was apparent in the kidney or brain following administration of dexamethasone to rats similarly to the observations following Cd administration. OST mRNA was reduced in the NRK cells by dexamethasone, while no alterations were observed in the kidney or brain. On the other hand, Cd which showed no inductive effect in NRK cells, significantly induced OST mRNA expression in the kidney and brain. The rat OST promoter has not yet been cloned, while bovine[20] and murine[10] OST promoters are known to contain MRE alone and both MRE and GRE, respectively. It has been reported that OST mRNA in rat tissues where MT mRNA is dominantly induced by Cd is not always inducible by Cd and *vice versa*.[9] It is suggested that the Cd level in cells is immediately affected by induction of MT and OST, which may cause transposition of Cd and alter availability of Cd to activate MRE. Administration of dexamethasone also caused preferential expression of MT mRNA and protein in the kidney and brain.[7] The increased MT protein level ecoked reductions in levels of endogenous stimulators including heavy metals and free radicals available to activate the SelP promoter. Thus, it is possible that direct stimulation of the GRE of the SelP promoter by dexamethasone was reduced by the decreasing stimulation of the stress promoter sequence through changes in the levels of stress-related stimulators and/or suppressors, resulting in no apparent alteration in the level of SelP mRNA. Differences in responses of OST expression to Cd in vitro and in vivo have also been reported, i.e. osteoligament cells did not respond to Cd[15] but OST mRNA in tissues increased after Cd administration to rats.[9] Since expression of MT mRNA was observed ubiquitously,[7] the induced MT might affect the availability of stress-related stimulators and/or suppressors in vivo even when the net increase in each tissue was not high.

It is also possible that SelP mRNA induction may occur in a regionally restricted manner in the brain and would not be apparent in the Northern blot analysis using RNA from the whole brain. In situ hybridization was performed to confirm regional expression of SelP, MT and OST mRNAs and to examine whether the effects of Cd and dexamethasone are consistent throughout the brain. Regional differences in expression of SelP and OST mRNAs were observed in the brain, while MT mRNA was expressed ubiquitously. No regional differences were detected in induction of MT mRNA, of which inducibility was the highest among the mRNAs examined, by Cd and dexamethasone. Regional differences in the induction of SelP and OST mRNAs were not obvious by in situ hybridization. In situ hybridization could not confirm whether dexamethasone altered SelP and OST mRNA expression in specific regions. However, the extremely high basal expression of SelP mRNA in the cerebellum observed by in situ hybridization suggested that SelP plays an

important role in this brain region, which is the target for the selenium toxicity known as "blind stagger".[6] Moreover, cerebellar dysfunction is one of the major symptoms of methyl-Hg intoxication, which is improved by selenium administration.[12,18] Constitutive expression of SelP in the cerebellum, even when it is not inducible, may play an important role not only in selenium homeostasis but also in detoxication of methyl-Hg.

Thus, expression of SelP mRNA is characterized by high basal expression and inducibility by GRE but not by MRE, while it is affected by other inducible proteins, namely MT and OST. Unfortunately, SelP displays no significant enzymatic activity and specific antibodies to this molecule are not yet available; hence, neither the dynamics nor the physiological function of SelP are yet known. Further investigations focusing on the expression and translation of SelP will help in elucidating such mechanisms.

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