Selenoprotein expression and brain development in preweanling selenium- and iodine-deficient rats

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ABSTRACT

Selenium deficiency causes further impairment of thyroid hormone metabolism in iodine-deficient rats and therefore could have a role in the aetiology of both myxoedematous and neurological cretinism in humans. Thyroidal type I iodothyronine deiodinase (ID-I), cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase activities were increased in iodine-deficient adult rats and their offspring at 11 days of age. Thyroidal ID-I activity was unchanged and thyroidal cytosolic glutathione peroxidase activity was decreased by more than 75% by combined selenium and iodine deficiency in 11-day-old rats, indicating that, while the thyroid retained an ability to produce 3,3',5-tri-iodothyronine (T₃), the gland was probably more susceptible to peroxidative damage caused by increased hydrogen peroxide concentrations driven by increased thyrotrophin. Thyroidal atrophy, common in myxoedematous cretinism, did not occur in iodine- or selenium and iodine-deficient rat

pups. Iodine deficiency increased brain type II iodothyronine deiodinase activity 1.5-fold in 4-day-old rats and 3-fold in 11-day-old rats, regardless of selenium status. Thus rats were able to activate compensatory mechanisms in brain that would maintain T₃ concentrations in selenium and iodine deficiencies. Surprisingly, however, selenium deficiency had a greater effect than iodine deficiency on markers of brain development in rat pups. Expression of the brain-derived neurotrophic factor (BDNF) mRNA was decreased in selenium deficiency in 4- and 11-day-old pups and in combined selenium and iodine deficiency in 4-day-old pups. Iodine deficiency caused an increase in BDNF expression in 11-day-old pups but had no effect on 4-day-old pups. Myelin basic protein mRNA expression in brain was decreased by combined selenium and iodine deficiency in 11-day-old rats.

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INTRODUCTION

Selenium and iodine are both essential for thyroid hormone metabolism and thus play a vital role in the health of man and animals. Iodine is an essential substrate for thyroid hormone synthesis, whilst selenium is required for the expression of the selenoenzymes type I iodothyronine deiodinase (ID-I) and type II iodothyronine deiodinase (ID-II), which are crucial in the generation of the active hormone 3,3',5-tri-iodothyronine (T₃) (Arthur *et al.* 1990a, Croteau *et al.* 1995). A consequence of selenium deficiency is that conversion of thyroxine (T₄) to T₃ by hepatic and renal ID-I is decreased by over 90%. In contrast, thyroidal ID-I activity is increased in acute selenium deficiency which helps maintain plasma

T₃ concentrations (Beckett *et al.* 1993). Further compensatory mechanisms in selenium deficiency include no change in brain ID-II activity in rats until very severe deficiency is induced. This allows maintenance of T₃ concentrations within the central nervous system, which cannot utilise circulating T₃ (Beckett *et al.* 1993, Mitchell *et al.* 1996).

Mammals depend on thyroid hormones for normal growth and development, with nuclear T₃ receptors detected in foetal rat brain by 13 days of gestation (Perez-Castillo *et al.* 1985). In iodine deficiency, a number of mechanisms are evoked to maintain thyroid hormone synthesis. Such mechanisms also increase the requirement for selenoproteins in the thyroid gland (Mitchell *et al.* 1996). Selenium deficiency may therefore aggravate the damaging effects of iodine deficiency in man and

animals by increasing the hypothyroid stress on the thyroid gland (Beckett *et al.* 1993, Contempre *et al.* 1995). In contrast, selenium deficiency can provide partial protection against the lowering of plasma thyroxine (T_4) concentrations in humans, which may help to prevent foetal brain damage and neurological cretinism (Contempre *et al.* 1991, 1992).

Evidence would thus suggest that concurrent selenium deficiency in adult iodine-deficient rats causes a greater oxidant stress on the thyroid gland than iodine deficiency alone, yet sufficient selenium is 'channelled' towards the thyroid gland to try to maintain the thyroidal selenoprotein activity (Beckett *et al.* 1993, Mitchell *et al.* 1996). Consequently, we examined the effects of concurrent selenium and iodine deficiencies on adult rats and their offspring at different postnatal ages to determine development of the ability of preweanling rats to retain thyroid hormone metabolism and function. We also assessed brain developmental biochemistry of pups of selenium- and iodine-deficient rats by the expression of the brain-derived nerve growth factor (BDNF) (Leibrock *et al.* 1989) and the myelination marker, myelin basic protein (MBP) (Kristensson *et al.* 1986).

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, chemicals were purchased from Sigma Chemical Company, Poole, Dorset, UK. Multiprime labelling kits, Hyperfilm-MP and [^{32}P]dCTP for mRNA determination along with ^{125}I -labelled reverse T_3 for deiodinase determination were purchased from Amersham International, Amersham, Bucks, UK.

Animals and diets

Groups of three female Hooded Lister weanling rats of the Rowett Institute strain were fed on one of the four diets: (1) selenium- and iodine-adequate control (+Se+I), (2) selenium-deficient iodine-adequate (−Se), (3) iodine-deficient selenium-adequate (−I), (4) selenium- and iodine-deficient (−Se−I) (Mitchell *et al.* 1996). After 8 weeks the rats were mated with normal males. At the end of the experiment, rats were anaesthetised with isoflurane and blood collected by cardiac puncture from adult and 11-day-old rats. Pups from each mother were killed on postnatal days 1, 4 and 11. Blood from 11-day-old animals was pooled in each

litter to allow sufficient sample to harvest plasma. Liver, thyroid and brain were removed, immediately frozen in liquid nitrogen and stored at -70°C before analysis.

Enzyme activities and thyroid hormone concentrations

ID-I and ID-II activities were determined using ^{125}I -labelled reverse T_3 as substrate (Nicol *et al.* 1994). Cytosolic glutathione peroxidase (cGSHPx) activity was measured by the method described by Beckett *et al.* (1987) and phospholipid hydroperoxide glutathione peroxidase (phGSHPx) activity by the method of Weitzel *et al.* (1990). Plasma thyrotrophin-stimulating hormone (TSH), T_4 and T_3 concentrations were determined as described by Arthur *et al.* (1990b).

Northern blot hybridisation

Total RNA was extracted from tissues (Chomczynski & Sacchi 1987) and 20 μg aliquots of mRNA separated by electrophoresis on a 1.2% denaturing agarose gel. The separated RNA was transferred to nylon membrane by capillary blotting and fixed using UV-light-induced cross-linking. cDNA probes for ID-I, cGSHPx, phGSHPx and 18S rRNA were as described previously (Mitchell *et al.* 1996). The 2.21 kb MBP cDNA was kindly provided by Dr Robert Lazzarini, The Mount Sinai School of Medicine (Kristensson *et al.* 1986). The partial BDNF clone was provided by Dr Lisa Monteggia, Abbott Laboratories, IL, USA (Giordano *et al.* 1992). Hybridisation was performed as described previously (Mitchell *et al.* 1996). mRNA was quantified using a Packard Instantimager and by autoradiography against Hyperfilm-MP at -70°C . After use of each cDNA probe, membranes were washed in 0.1% SDS at 95°C for 10 min to remove the label before rehybridisation. mRNA levels are given as ^{32}P counts corrected for hybridisation with 18S rRNA to correct for any variation in loading or transfer to the membrane. Results for each filter were expressed as a percentage of the mean for animals fed on the control diet (+Se+I).

Statistical analysis

Group means were compared by ANOVA using the 'Genstat Statistics Package' (Genstat 5 Committee, Genstat 5 release3, reference manual 1993, Clarendon, Oxford, UK).

TABLE 1. Effects of selenium and iodine deficiencies on body and thyroid gland weights and plasma thyroid hormone concentrations. Data are means $\pm$ S.E.M (adults $n=3$, pups $n=5$)

	+Se+I	-Se	-I	-Se-I
Body weight (g)				
Adult-mating	206 $\pm$ 8.30	187 $\pm$ 5.60	191 $\pm$ 5.13	186 $\pm$ 5.52
Adult-final	249 $\pm$ 2.60	226 $\pm$ 4.51*	222 $\pm$ 6.68**	218 $\pm$ 6.84**
11-day-old	22.5 $\pm$ 2.30	23.0 $\pm$ 0.76	19.2 $\pm$ 0.35	20.3 $\pm$ 1.05
4-day-old	7.83 $\pm$ 0.66	8.62 $\pm$ 0.45	7.84 $\pm$ 0.34	7.41 $\pm$ 0.28
1-day-old	5.75 $\pm$ 0.72	4.95 $\pm$ 0.53	4.79 $\pm$ 0.25*	4.07 $\pm$ 0.43***
Average pups/dam	8	8	9	7
Thyroid weight (% body weight)				
Adult	0.011 $\pm$ 0.001	0.012 $\pm$ 0.001	0.063 $\pm$ 0.005***	0.073 $\pm$ 0.008***
11-day-old	0.022 $\pm$ 0.003	0.019 $\pm$ 0.001	0.055 $\pm$ 0.008***	0.056 $\pm$ 0.005***
Plasma T₄ (nmol/l)				
Adult	39.0 $\pm$ 6.08	48.3 $\pm$ 4.33	5.75 $\pm$ 1.32***	7.67 $\pm$ 0.33***
11-day-old	51.3 $\pm$ 2.73	66.7 $\pm$ 2.85**	10.3 $\pm$ 1.38***	9.7 $\pm$ 0.5***
Plasma T₃ (nmol/l)				
Adult	0.46 $\pm$ 0.012	0.51 $\pm$ 0.062	0.24 $\pm$ 0.060***	0.41 $\pm$ 0.078
11-day-old	0.83 $\pm$ 0.13	0.73 $\pm$ 0.035	0.62 $\pm$ 0.06*	0.58 $\pm$ 0.10*
Plasma TSH (ng/ml)				
Adult	1.94 $\pm$ 0.43	4.04 $\pm$ 0.20*	51.1 $\pm$ 4.23***	58.6 $\pm$ 6.04***
11-day-old	3.67 $\pm$ 0.24	4.01 $\pm$ 0.39	21.82 $\pm$ 2.89***	14.19 $\pm$ 0.67***

Thyroid gland weights are expressed as percentage of total body weight.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared with control group (+Se+I) (ANOVA).

RESULTS

Rat body and thyroid weights

There were no significant differences between the groups of female rats with respect to food intake (not shown), weight at mating or average number of pups born (Table 1). However, both selenium and iodine deficiencies caused decreases in body weight of the female rats at the end of the experiment. Pups from iodine-deficient females were of lower body weight than controls at 1 day old but there was no effect of selenium or iodine deficiencies on body weights of 4- or 11-day-old pups (Table 1). Selenium deficiency had no effect on thyroid weight in adult rats or their 11-day-old pups (expressed as percentage of body weight). Iodine deficiency caused 7-fold and 2.5-fold increases in thyroid weight in adult rats and their 11-day-old pups, regardless of selenium status (Table 1).

Plasma thyroid hormone concentrations

In selenium deficiency, plasma T₄ concentrations were unchanged in adults but increased significantly in 11-day-old pups (Table 1). Iodine deficiency caused decreases in plasma T₄ concentrations to <20% of control (+Se+I) in adult and 11-day-old

pups, regardless of selenium status. Plasma T₃ concentrations were unaffected by selenium deficiency but were decreased by iodine deficiency in adult and 11-day-old pups. Combined selenium and iodine deficiency decreased plasma T₃ concentrations in 11-day-old rats but had no effect in adult rats. Iodine deficiency caused 25-fold and 5-fold increases in plasma TSH concentration in adult rats and their 11-day-old pups, regardless of selenium status. Plasma TSH concentrations were increased by selenium deficiency in adult rats but were unchanged in 11-day-old pups.

Thyroid selenoenzyme activities

In adult rats, thyroid ID-I activity (per mg protein) was increased in selenium deficiency, decreased in iodine deficiency and unchanged in combined selenium and iodine deficiency (Table 2). However, total ID-I activity per gland was increased 2-fold in both iodine-deficient groups and unchanged in selenium deficiency (Fig. 1). ID-I activity in thyroid glands from 11-day-old rats (per gland and per mg protein) was decreased by selenium deficiency, increased by iodine deficiency and unchanged by combined selenium and iodine deficiency (Fig. 1 and Table 2). In adult rats, thyroidal cGSHPx and

TABLE 2. Hepatic and thyroidal selenoenzyme activities in adult rats and their offspring at 1, 4 and 11 days of age. Group means ± s.e.m. are shown (adults *n*=3, pups *n*=5)

	Enzyme activity			
	+ Se+I	– Se	– I	– Se – I
Adult thyroid				
ID-I	65.1 ± 9.02	83.2 ± 2.77**	51.9 ± 2.89*	58.4 ± 8.46
cGSHPx	0.12 ± 0.01	0.08 ± 0.01**	0.24 ± 0.02***	0.15 ± 0.02
phGSHPx	3.07 ± 0.16	2.30 ± 0.60*	3.66 ± 0.14*	3.17 ± 0.19
11-Day thyroid				
ID-I	29.7 ± 8.29	11.4 ± 0.70***	69.4 ± 9.62***	30.8 ± 4.84
cGSHPx	0.05 ± 0.01	0.012 ± 0.01***	0.08 ± 0.01*	0.02 ± 0.01***
phGSHPx	1.30 ± 0.20	0.80 ± 0.02**	1.63 ± 0.18	1.40 ± 0.15
Adult liver				
ID-I	26.1 ± 1.92	2.43 ± 0.49***	4.8 ± 0.43***	1.6 ± 0.44***
cGSHPx	0.89 ± 0.04	0.018 ± 0.01***	0.77 ± 0.070	0.022 ± 0.002***
phGSHPx	1.01 ± 0.20	0.51 ± 0.16*	1.16 ± 0.16	0.32 ± 0.17***
11-Day liver				
ID-I	26.6 ± 2.54	1.5 ± 0.40***	16.4 ± 1.01**	0.98 ± 0.14***
cGSHPx	0.16 ± 0.021	0.01 ± 0.001***	0.18 ± 0.034	0.01 ± 0.001***
phGSHPx	1.15 ± 0.40	0.27 ± 0.06***	0.46 ± 0.07**	0.43 ± 0.12**
4-Day liver				
ID-I	17.5 ± 2.69	1.5 ± 0.22***	11.3 ± 0.89*	1.7 ± 0.44***
cGSHPx	0.12 ± 0.005	0.01 ± 0.001***	0.13 ± 0.018	0.01 ± 0.001***
phGSHPx	0.71 ± 0.17	0.39 ± 0.19*	0.69 ± 0.17	0.15 ± 0.04***
1-Day liver				
ID-I	13.9 ± 2.11	1.4 ± 0.38***	13.1 ± 1.15	1.5 ± 0.44***
cGSHPx	0.19 ± 0.021	0.01 ± 0.001***	0.15 ± 0.018	0.01 ± 0.001***
phGSHPx	2.08 ± 0.52	1.10 ± 0.46*	1.41 ± 0.23*	1.26 ± 0.35*

ID-I activity is expressed as pmol I released/min per mg protein. cGSHPx activity is expressed as units/mg protein and phGSHPx activity is expressed as munits/mg protein.
P*<0.05, *P*<0.01, ****P*<0.001 as compared with control (+Se+I) (ANOVA).

phGSHPx activities were decreased by selenium deficiency, increased by iodine deficiency and unchanged in combined selenium and iodine deficiency in adult rats (Table 2). cGSHPx activity in thyroids of 11-day-old rats was increased in iodine deficiency and decreased in both selenium-deficient groups. Thyroidal phGSHPx activity in 11-day-old rats was decreased in selenium deficiency but unchanged in both iodine-deficient groups.

Hepatic selenoenzyme activities

Selenium deficiency, regardless of iodine status, decreased hepatic ID-I activity to less than 10% of control (+Se+I) in adult rats and their offspring at 1, 4 and 11 days (Table 2). Iodine deficiency decreased hepatic ID-I activity in adult, 11-day- and 4-day-old rats but had no effect on ID-I activity in livers from 1-day-old rats. Selenium deficiency

FIGURE 1. ID-I activities in thyroid glands from adult and 11-day-old rats. ID-I activity is expressed as pmol iodine released/gland. Means ± s.e.m. are shown (adults *n*=3, pups *n*=5). ***P*<0.01, ****P*<0.001 as compared with control (+Se+I) (ANOVA).

TABLE 3. Selenoperoxidase activity and mRNA abundance in cerebrum from 1-, 4- and 11-day-old rats, expressed as % control where control value=100%. Mean $\pm$ S.E.M. are shown. ($n=5$ /group)

	+Se+I	- Se	- I	- Se - I
11-Day-old				
<i>c</i> GSHPx activity	100 $\pm$ 4.5	51.9 $\pm$ 3.5**	89.1 $\pm$ 7.9	56.7 $\pm$ 5.9**
<i>c</i> GSHPx mRNA	100 $\pm$ 8.7	156 $\pm$ 13.0**	139 $\pm$ 8.7*	148 $\pm$ 21.7*
<i>ph</i> GSHPx activity	100 $\pm$ 8.9	107 $\pm$ 18.5	97.4 $\pm$ 10.5	89.9 $\pm$ 8.1
<i>ph</i> GSHPx mRNA	100 $\pm$ 3.8	169 $\pm$ 15.3***	130 $\pm$ 3.8**	119 $\pm$ 11.5*
4-Day-old				
<i>c</i> GSHPx activity	100 $\pm$ 4.8	52.1 $\pm$ 6.9**	92.0 $\pm$ 2.9	59.2 $\pm$ 4.4**
<i>c</i> GSHPx mRNA	100 $\pm$ 17.0	61.7 $\pm$ 4.3*	79.6 $\pm$ 6.4	70.2 $\pm$ 8.5*
<i>ph</i> GSHPx activity	100 $\pm$ 7.9	94.8 $\pm$ 1.9	102 $\pm$ 7.7	107 $\pm$ 7.8
<i>ph</i> GSHPx mRNA	100 $\pm$ 10.8	132 $\pm$ 8.1**	100 $\pm$ 8.1	124 $\pm$ 10.8*
1-Day-old				
<i>c</i> GSHPx activity	100 $\pm$ 7.3	41.2 $\pm$ 3.1***	72.6 $\pm$ 3.1*	48.4 $\pm$ 5.3**
<i>c</i> GSHPx mRNA	100 $\pm$ 8.5	74.8 $\pm$ 9.4*	63.5 $\pm$ 9.8*	74.2 $\pm$ 7.0*
<i>ph</i> GSHPx activity	100 $\pm$ 38.3	104 $\pm$ 14.6	127 $\pm$ 18.1	107 $\pm$ 9.9
<i>ph</i> GSHPx mRNA	100 $\pm$ 13.2	127 $\pm$ 4.1	60.2 $\pm$ 10.2**	105 $\pm$ 18.4

*c*GSHPx activity is expressed as units/mg protein and *ph*GSHPx activity as munits/mg protein. 100% *c*GSHPx activity is 0.064, 0.048 and 0.040 units/mg protein in 1-, 4- and 11-day-old rats. 100% *ph*GSHPx activity is 0.95, 4.3 and 4.0 munits/mg protein in 1-, 4- and 11-day-old rats.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared with control (+Se+I) (ANOVA).

and combined selenium and iodine deficiency caused decreases in hepatic *c*GSHPx activity to less than 5% of control (+Se+I) in adult rats and their offspring at 1, 4 and 11 days old. Iodine deficiency had no effect on hepatic *c*GSHPx activity in adult rats or their pups. Selenium deficiency decreased hepatic *ph*GSHPx activity in adult, 11-day-, 4-day- and 1-day-old rats, regardless of the iodine status. Iodine deficiency alone caused decreases in hepatic *ph*GSHPx activity in 1- and 11-day-old rats but had no effect in adult and 4-day-old pups. Hepatic ID-I, *c*GSHPx and *ph*GSHPx mRNA levels reflected the enzyme activities in adult, 11-day- and 4-day-old rats (not shown). mRNA levels could not be determined in livers from 1-day-old animals because of sample degradation.

Brain selenoenzyme activities and mRNA expression

Whole brain ID-I activity was unchanged by selenium and iodine deficiencies in 11- and 4-day-old rats but was increased by combined selenium and iodine deficiency in 1-day-old animals (Fig. 2). ID-II activity was increased in both iodine-deficient groups in 11- and 4-day-old animals and was decreased by selenium deficiency in 11-day-old rats. Brain ID-II activity was less than 0.01 fmol iodine released/min per mg protein in 1-day-old rats and thus could not be

determined. Brain *c*GSHPx activity and mRNA levels were decreased by selenium and iodine deficiencies in 1-day-old rats and in both selenium-deficient groups in 4-day-old rats (Table 3). Selenium deficiency decreased brain *c*GSHPx activity in 11-day-old rats despite mRNA levels being increased by selenium and iodine deficiencies. Brain *ph*GSHPx activities were unchanged by selenium and/or iodine deficiencies in 1-, 4- and 11-day-old rats. Brain *ph*GSHPx mRNA levels were increased by selenium deficiency in 1-day-old rats and in both selenium-deficient groups in 4- and 11-day-old rats. Iodine deficiency decreased, did not affect and increased cerebral *ph*GSHPx mRNA levels in 1-, 4- and 11-day-old rats respectively (Table 3).

Brain BDNF and MBP expression

Brain BDNF mRNA expression was decreased by selenium deficiency in 4- and 11-day-old rats (Fig. 3). Iodine deficiency caused an increase in BDNF mRNA levels in 11-day-old rats but had no effect on expression in brain of 4-day-old rats. Combined selenium and iodine deficiency decreased cerebral BDNF mRNA levels in 4-day-old rats but had no effect in 11-day-old rats. MBP mRNA was not detected in the brain of 4-day-old rats. In brains from 11-day-old rats, MBP expression was lower in combined selenium- and iodine-deficient pups (Fig. 4).

FIGURE 2. ID-I (top) and ID-II (bottom) activities in cerebrium from 1-, 4- and 11-day-old rats. ID-I activity is expressed as pmol iodine released/h per mg protein. ID-II activity is expressed as fmol iodine released/h per mg protein. N.D., not determined. Means $\pm$ S.E.M. are shown ($n=5$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared with control (+Se+I) (ANOVA).

DISCUSSION

In adult rats and their 19-day-old pups, compensatory mechanisms exist to minimise thyroid hormone imbalance in selenium and iodine deficiencies (Mitchell *et al.* 1996, 1997). However, prolonged selenium and iodine deficiencies can have an adverse effect on brain biochemistry and development (Meinhold *et al.* 1993). This paper shows that such compensatory mechanisms exist, but are less efficient in 1-, 4- and 11-day-old selenium- and iodine-deficient rats. The diets used

FIGURE 3. Effect of selenium and iodine deficiencies on cerebral BDNF mRNA expression in 4- and 11-day-old rats. mRNA was quantified using a Packard Instantimager and expressed as % control (+Se+I) where control value=100%. Means $\pm$ S.E.M. are shown ($n=5$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared with control (+Se+I) (ANOVA).

FIGURE 4. Cerebral MBP mRNA expression in 11-day-old rats. For experimental details see Fig. 2. Means $\pm$ S.E.M. are shown ($n=5$). ** $P<0.01$ as compared with control (+Se+I) (ANOVA).

in the present studies caused iodine deficiency, as is evident from thyroid hypertrophy (7-fold increase in weight) and increased thyroidal ID-I activity in adult rats regardless of selenium status. In 11-day-old rats, thyroid gland weights increased 2.5-fold, and ID-I activity in the gland was increased by iodine deficiency alone, but was unchanged with a concurrent selenium deficiency and decreased by more than 50% in selenium deficiency alone (Fig. 1, Table 2). Thyroidal *c*GSHPx activity, which in 19-day-old animals was unchanged in combined selenium and iodine deficiency (Mitchell *et al.* 1996), is decreased by more than half in 11-day-old rats, probably making the gland more susceptible to peroxidative damage (Table 2). Plasma TSH concentrations were increased in both iodine-deficient groups in adults and 11-day-old rats, but T_4 concentrations were less than 20% of control levels, and plasma T_3 concentrations were decreased. Hepatic ID-I, *c*GSHPx and *ph*GSHPx activities were decreased by selenium deficiency in adult rats and their pups at 1, 4 and 11 days, as the liver does not retain selenium as well as other organs (Mitchell *et al.* 1996). The above results are consistent with the compensatory mechanisms in selenium-deficient 1-, 4- and 11-day-old rats including loss of selenium from tissues such as liver and muscle.

In contrast with changes in liver selenoenzymes, brain ID-I activities in 4- and 11-day-old pups were unaffected by selenium or iodine deficiencies and were increased in 1-day-old pups in the concurrent selenium and iodine deficiency group (Fig. 2). Brain *ph*GSHPx activities were not changed in 1-, 4- and 11-day-old rats probably because increased mRNA counteracted decreased selenium availability; however, *c*GSHPx activity was decreased despite increases in mRNA levels (Table 3). A similar preferential supply of selenium to *ph*GSHPx over *c*GSHPx occurs in different brain regions from adult rats and their 19-day-old pups (Mitchell *et al.* 1997). The brains from the younger rats were, however, too small to divide into the regions used in previous experiments (Mitchell *et al.* 1997). Brain ID-II activity was unaffected by selenium deficiency in 4-day-old animals but decreased by more than 50% in 11-day-old animals as compared with selenium-adequate controls. This would suggest that the older animals have become more selenium-deficient despite the fact that the brain retains the micronutrient at the expense of other organs. Alternatively, there could be a form of ID-II which does not contain selenium but is specific to brain. Brain ID-II activity is also decreased

in third-generation selenium-deficient adult rats; however, tissue T_3 concentrations are not affected (Campos-Barros *et al.* 1997). Brain ID-II activity was increased 1.5-fold and 3-fold in iodine deficiency in 4- and 11-day-old rats respectively regardless of selenium status (Fig. 2). In addition, brain ID-II activity increases substantially in iodine-deficient adult rats (Mitchell *et al.* 1997), but, despite this, tissue T_3 concentrations are decreased (Campos-Barros *et al.* 1997). Selenium deficiency caused 25 and 50% decreases in BDNF mRNA expression in 4- and 11-day-old rats respectively (Fig. 3). However, the differing effects of iodine deficiency on BDNF expression make these results very complicated to interpret. Although localised changes in thyroid hormone metabolism could have influenced BDNF expression, other selenoproteins such as thioredoxin reductase, which can indirectly modulate gene expression, may underlie the altered gene expression caused by selenium deficiency (Gladyshev *et al.* 1996, McLeod *et al.* 1997).

Myelination in the rat begins at around 10 days after birth and is delayed by hypothyroidism, as assessed by determination of MBP gene expression (Kristensson *et al.* 1986, Dussault & Ruel 1987). In this experiment cerebral MBP mRNA abundance was lower in combined selenium and iodine deficiency than in the single micronutrient deficiencies or control animals. Thus a concurrent selenium deficiency may cause further delay in brain development in T_4 -deficient animals.

From birth, rats have compensatory mechanisms which can protect against a decrease in brain T_3 concentrations induced by selenium and iodine deficiencies. However, these mechanisms do not confer complete protection against the effects of selenium and/or iodine deficiencies, as is evident from decreases in MBP and BDNF mRNAs. The effects of the deficiencies on these mRNAs were different, which indicates that the mechanisms causing the changes are probably independent. Thus selenium may influence gene expression in ways that are not always mediated by thyroid hormone metabolism.

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