Acute iodine ingestion increases intrathyroidal glutathione

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ABSTRACT. In genetically predisposed individuals, autoimmune lymphocytic thyroiditis (LT) is potentiated by excess dietary iodine (I). There have been data which suggest that oxidative stress may have a role in iodine-induced LT. These in vivo studies were undertaken to examine the effect of iodine on intrathyroidal levels of the potent antioxidant glutathione (GSH) and see if the thyroids of LT-prone BB/Wor rats have aberrant GSH responses after iodine-loading. LT-prone BB/Wor, non LT-prone BB/Wor and Wistar rats were randomized to receive either 0.05% I (as NaI) or tap water. Thyroid and liver homogenates were assayed individually for GSH. Following the administration of 0.05% iodine water overnight, all of the animals demonstrated a rise in intrathyroidal GSH regardless of LT-proneness. To determine whether this was a dose-dependent response, Wis rats were randomized to receive tap, 0.0125%, 0.025%, 0.05%, or 0.075% I, overnight. Intrathyroidal GSH levels rose with increasing iodine concentrations peaking at 0.025% I. Hepatic GSH levels were unaltered by iodine treatment. Ten days of 0.05% I water did not result in any difference between the GSH levels of thyroids from treated and control rats. Frozen sections of the thyroids and livers from iodine-treated rats were compared to tap-water controls after staining with Mercury Orange for GSH and Schiff's reagent for evidence of lipid peroxidation. Iodine-treated thyroids had an apparent shift of GSH staining from the apical border to the cytoplasm. However, there was no Schiff's staining indicative of lipid peroxidation in the iodine-treated thyroids. These results show that acute iodine loads increase intrathyroidal GSH and LT-prone rats do not have an inherent deficit in this response. Therefore, we conclude that there is no role for oxidative damage in iodine-induced LT. We propose that GSH has a role in intrathyroidal iodine metabolism and thyroid hormone synthesis.

INTRODUCTION

Excess iodine may affect the thyroid in one of four ways (1, 2). 1) Low levels of iodine increase the gland's uptake of the iodide anion (I-). 2) Large amounts of circulating iodine inhibit the release of thyroid hormone. This effect may be attributed to the prevention of thyroglobulin (Tg) proteolysis. 3) Extrathyroidal iodide concentrations in excess of 20 - 35 μg % inhibit the incorporation of I into thyroglobulin (Wolff-Chaikoff effect). 4) Extrathyroidal iodide concentrations exceeding 3 x 10^-5 M may prevent intrathyroidal I transport. However, the mechanism by which excess dietary iodine potentiates the development of lymphocytic thyroiditis (LT) is still unknown (3). Lymphocytic thyroiditis has an increased incidence among populations with high dietary iodine intake (3-7). Animal models for spontaneous and iodine-exacerbated LT include the BB/Wor rat, Obese strain (OS) chicken, dog and thymectomized Buffalo rat (3,8-14). In Japan, many cases of LT improved after dietary iodine levels were reduced (6). When BB/Wor rats and OS chickens are fed low iodine (Remington) diets, the incidence of LT is significantly reduced (10, 12). At the present time, there are two main theories proposing the mechanism by which iodine facilitates the appearance of LT (3). One is that increased iodination of thyroglobulin (Tg) by excess I makes Tg more antigenic (15, 16). The other is that excess oxidized species of I (I^0, I_3, I^+, IO^-) precipitate oxidative damage (17, 18). Many et al. have recently published data demonstrating the toxic effects of acute exposure to high iodine concentrations in cultured human thyroid follicles. They propose that these effects are due to iodine-induced free radical production and oxidative damage (18). We have previously reported that acute dietary iodine-loading does not elevate intrathyroidal malondialdehyde levels, a sensitive biochemical indicator of lipid per-

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oxidation and oxidative damage, in rat thyroids (19).
The tripeptide thiol glutathione (GSH) (gamma-gluc­
cys-gly), is one of the most important intracellular defenses against oxidative stress in eukaryotic cells (20,21). If excess dietary iodine precipitates ox­
idative damage, it should be reflected in the GSH levels (20). A compromised GSH response might account for susceptibility to iodine-induced injury in LT-prone individuals because in the absence of an adequate antioxidant, oxidative stress could ini­
tiate an inflammatory reaction which is then per­
petuated by an intolerant immune system. In the present studies, we have examined the effect of ex­
cess dietary iodine on intrathyroidal GSH levels in
LT-prone BB/Wor and non LT-prone rats.

MATERIALS AND METHODS
Experimental animals
LT-prone BB/Wor rats develop spontaneous LT and
diabetes mellitus (DM) (8). The NB-line has nearly a
100% incidence of LT by 120 days of age but
lymphocytic infiltration is rare before 60 days of age
(9-11). NB line LT-Prone (LT-P) and WA line LT-
Resistant (LT-Res) BB/Wor rats were obtained from
the University of Massachusetts Medical Center
breeding colony (Worcester, MA) and Wistar Furth
(Wis) rats were obtained from the Charles River
BioBreeding Facility (Wilmington, MA). Since we
were investigating the predisposition to LT, we want­
ed to avoid confounding results. Therefore, all of the
rats were studied at 6-8 weeks of age, which is be­
fore the time spontaneous LT and DM are expected
to occur in the LT-P rats. LT-P rats were tested for
glucosuria twice weekly with Tes-Tape (Eli Lilly Co.,
Indianapolis, IN). No rats developed DM.

The effect of iodine on GSH levels
In separate studies, 30 LT-P, 12 LT-Res, and 30
Wis rats were fed standard rat chow and random­
ized to receive either tap or 0.05% I (as NaI) water
overnight prior to euthanasia the following morning.
Overnight, each rat ingested approximately 25 ml of
water. Therefore, the iodine group received ap­
proximately 12.5 mg I. We have previously found
that this level of iodine ingestion results in serum
iodine levels in excess of the 20 - 35 f.lg % neces­
sary for the Wolff-Chaikoff effect to occur (1,14). To
study the effect of incremental doses of iodide, 20
Wis rats were randomized to receive tap, 0.0125%
I, 0.025% I, 0.05% I, or 0.075% I water overnight. To
study the effect of prolonged iodine ingestion, 16
Wis rats were randomized to receive tap or 0.05%
I water for 10 days before euthanasia.

Animals were euthanized by cervical dislocation af­
after ketamine anesthesia. The livers and thyroids
were removed and placed on ice in nitrogen-sup­
plemented Hank’s Balanced Salt Solution (to pre­
vent autooxidation). GSH assays of individual thy­
roid and liver homogenates were performed in trip­
llicate according to the method of Sedlak and
Lindsay for measurement of non protein thiols (22).
Standard curves were generated from known con­
centrations of reduced GSH (Sigma, St. Louis, MO,
USA). Care was taken to ensure that the final pH of
the assay mixture was between 8.0 and 9.0. Color
development is inconsistent and nonlinear in rela­
tionship to GSH at pH’s below 8.0 or above 9.0 and
oxidized GSH (GSSG) is favored at higher pH’s
(23). The interassay variability was 1.9%. Protein
concentrations were measured according to Lowry
(24).

The effect of iodine on the localization of
intrathyroidal GSH
Wis rats were randomized to receive either tap or
0.05% I water, overnight. The next morning, the an­
imals were euthanized and the thyroids and livers
removed. These tissues were fixed and stained with
Mercury Orange as described by Forkert and
Moussa (25). During short incubations, Mercury
Orange preferentially stains the thiol groups in GSH
(25).

The effect of iodine on lipid peroxidation
To examine whether acute iodine ingestion precip­
itated histologic evidence of oxidative damage, Wis
rats were randomized to receive either tap or 0.05%
I water, overnight. At the end of that time, they were
euthanized and the thyroids removed. These
glands were then stained with fresh Schiff’s reagent
as described by Pompella et al. (26). Schiff’s
reagent stains aldehyde functions that develop dur­
ing lipid peroxidation, a consequence of oxidative
damage (26).

Statistical analysis
GSH comparisons between the three rat lines were
done by ANOVA with Bonferroni’s correction for
multiple comparisons. Comparisons between two
groups were done by Student's t test.

RESULTS
Intrathyroidal GSH levels were higher in iodine-
treated rats regardless of LT-proneness [LT-P Tap:
24.7±4.0 nmol GSH/mg protein (mean±SE) vs LT-
P I: 31.5±1.4, Student’s t test p<0.002; Wis Tap:
27.5±1.5 vs Wis I: 34.9±1.5, p<0.002]. Failure to