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Taurine depletion increases phosphorylation of a specific protein in the rat retina

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Summary. Partial depletion of the taurine content in the rat retina was accomplished for up to 22 weeks by introduction of 1.5% guanidinoethanesulfonate (GES) in the drinking water. Taurine levels decreased by 50% after 1 week of GES treatment and by 80% at 16 weeks. Replacement of GES by taurine to the GES-treated rats from week 16 to 22 returned their taurine content to the control value. Whereas addition of taurine (1.5%) to the drinking water of control rats from week 16 to 22 elevated the retinal taurine content to 118% of the control value, the administration of untreated water to GES-treated animals for the 16 to 22 week time period increased the retinal taurine content to only 76% of the control value.

The amplitude of the electroretinogram (ERG) b-wave was decreased by 60% after GES-treatment for 16 weeks and maintained this reduced level for up to 22 weeks. Administration of taurine in the drinking water from week 16 to 22 returned the b-wave amplitude to a range not statistically different from the control values whereas the administration of untreated water produced less improvement.

After 6 weeks of GES treatment when the retinal taurine content was reduced by 70% and the amplitude of the b-wave was reduced by 50% (extrapolated from Figure 1), phosphorylation of a specific protein with an approximate molecular weight of 20K was increased by 94%. The increased phosphorylation of the ~20K protein observed after GES treatment was reversed when the animals were treated with taurine (1½%) in the drinking water for an additional 6 weeks. There was no change in the phosphorylation of the ~20K protein when animals were treated with taurine for 6 weeks. The data obtained support the theory that taurine may have a regulatory effect on retinal protein phosphorylation.

Keywords: Amino acids – Taurine – Taurine-depletion – Rat retina – Protein phosphorylation – Guanidinoethanesulfonic acid (GES) – Electroretinogram (ERG)

Introduction

Taurine (2-aminoethanesulfonic acid), an ubiquitous amino β-sulfonic acid found primarily in the animal kingdom, is a constituent of all mammalian tissues (Jacobsen and Smith, 1968). Evidence accumulated in the past few years suggests that taurine has a vital role in the physiology of excitable tissues such as the brain, heart, and retina (reviewed in Huxtable 1989, 1992; Lombardini, 1991). While some of the highest tissue contents of taurine are reported to be in the retina, attaining levels of 50μmoles/g wet tissue in the rat (Pasantes-Morates et al., 1972; Voaden et al., 1981; Gupta et al., 1981), there is at present no well-defined function or mechanism of action at the molecular level for taurine in this tissue (Lombardini, 1991). However, there is considerable evidence that taurine has a modulatory effect on calcium ion concentrations (Lombardini, 1991) and protein phosphorylation (Lombardini, 1991, 1992a,b, 1993, 1994) in various subcellular fractions of the retina. It is also well-recognized that taurine is an essential dietary component of the cat, for if this species is deprived of taurine, retinal degeneration occurs along with many other developmental pathologies (Sturman, 1993). The elegant studies of Sturman and colleagues have further demonstrated the nutritional need for taurine in the cat by observing morphologic damage in the tapetum, cerebellum and visual cortex in the taurine-deprived animal (Sturman, 1993).

Evidence for a vitamin-like role for taurine in species such as the rat has been delayed because of the ability of the rat to synthesize a portion of its body stores of taurine and not be solely dependent on dietary taurine (Huxtable et al., 1979, 1989). However, the rat can be partially depleted of its tissue stores of taurine by the addition of guanidinoethanesulfonate (GES) to the drinking water (Huxtable et al., 1979; Lombardini, 1981). GES, a structural analogue of taurine, depletes the taurine content of the various rat tissues 70–80% by inhibiting taurine transport (Huxtable et al., 1979; Lombardini, 1981). The use of GES as a taurine depletor has gained widespread acceptance in studying the role of taurine in the rat retina and both electrophysiological deficits and morphological changes (including the eventual frank degeneration of photoreceptors and shrinkage of the inner retinal layer) have been observed in these animals (Lake, 1981, 1982a,b, 1983, 1986, 1989; Lake and Malik, 1987; Pasantes-Morales et al., 1983; Quesada et al., 1984).

In the present study GES was added to the drinking water of rats with the objective being to investigate the effect of retinal taurine depletion on the phosphorylation of an $\sim$20K apparent molecular weight (M_r) protein present in the rat retina. Because previous studies have demonstrated that exogenous taurine has a significant inhibitory effect on the phosphorylation of this protein, it was hypothesized that one function of taurine may be to regulate retinal protein phosphorylation. In the present study, we tested this hypothesis by depleting taurine stores in the retinal tissue while examining phosphorylation activity. It seemed important, however, to distinguish between the effects on phosphorylation activity that result from the manipulation of the retinal taurine level and those that may result indirectly or

aberrantly as a consequence of frank degeneration of tissue. So, an essential prelude to the present study concerned whether our method of producing taurine depletion leads to any obvious sign of cell death or permanent functional deficit. This communication documents these effects.

Materials and methods

Materials

GES was synthesized according to the procedure of Morrison et al. (1958). Female Wistar rats weighing 50–75 grams at the start of the experiments were fed Purina rat chow and water *ad libitum*. The animals were housed in the vivarium and placed on a 12-h light-dark cycle (light cycle starting at 7:00 a.m.). Taurine was purchased from Sigma Chemical Co. [γ-^{32}P]ATP (30 Ci/mmole) was obtained from New England Nuclear Corporation.

Taurine determination

All animals were killed between 8:00 and 9:00 a.m. by anesthetizing with ether and then decapitation. The eyes were immediately removed from the animal and placed in 0.32 M sucrose (2°C). The retinal tissue was teased out of the eye cup with a small spatual and placed in a test tube with 0.5 ml of 0.1 N NaOH to solubilize the mixture. Protein content was determined on an aliquot of the solubilized preparation by the method of Lowry et al. (1951). The remaining solubilized retinal preparation was made acidic to pH 2.0 with 10% perchloric acid and then centrifuged for 10 minutes at 16,000 × g. Taurine content was determined on the protein-free supernatant with a Beckman amino acid analyzer using 0.2 N citrate buffer, pH 2.4.

Taurine depletion

Experimental animals were maintained on drinking water which contained 1.5% GES for the indicated times. Control animals were maintained on untreated water.

Electroretinogram (ERG) recording procedures

The rats, dark-adapted for 16 hours prior to testing, were anesthetized with sodium pentobarbital (30 mg/kg in 10% alcohol) which was administered intraperitoneally. Mydriacyl (1%) was used topically to dilate the pupils. The ERG electrodes were positioned on the rat under dim red light illumination. The eyelid was retracted. A cotton wick electrode (attached to a chlorided silver wire) was placed on the cornea. A second chlorided wire placed in a small cut in the cheek of the rat served as the indifferent electrode. A needle electrode was inserted in the rat's tail to provide the electrical ground connection. The stimulus was a Grass Xenon Flash Lamp placed approximately 6 inches from the eye. As the amplitude of the ERG is known to saturate with increasing flash intensity, ERGs evoked by high intensity flashes may not provide a sensitive index of pathophysiologic changes of the retina. Thus, in the present study, a very dim flash was used. The flash intensity selected was empirically determined in preliminary experiments. The lamp was set at its lowest intensity on the Grass stimulator and was further attenuated with several sheets of blue (Edmund Scientific, Barrington, NJ, #866) filter material until an ERG was obtained with roughly half the estimated maximal amplitude. Six sheets of the blue filter were eventually used. The resulting ERG was composed almost exclusively of a b-wave which was measured as the maximum positive excursion from the baseline. The recordings consist of a single ERG response, typically the first response obtained. The ERG was amplified with a Grass amplifier (P511H). Individual ERG responses were captured with a Nicolet 1072 averager with a 200 msec epoch and then

plotted on an X-Y plotter. The bandpass of the system was 0.3 to 300 Hz. The system was calibrated by placing a Grass calibrator at the input of the amplifier.

Preparation of the mitochondrial subcellular fraction

The mitochondrial fraction of the retinas was prepared as previously described (Lombardini, 1988). Briefly, 10–12 adult Wistar rats (175–225 g) were killed by decapitation. The eyes were immediately removed and placed in ice-cold 0.3 M mannitol, pH 7.4. The retinal tissue, maintained at ice temperatures for this and all subsequent procedures, was teased out of the eye cup and placed in the mannitol solution. The rod outer segments (ROSs) were removed by vortex-mixing the retina for 6 sec, allowing the tissue to settle, and then decanting (and discarding) the supernatant which contained the ROSs. The remaining tissue components were gently hand-homogenized with 10 up-and-down strokes in a Potter-Elvehjem homogenizer and centrifuged at 150 g for 15 min to remove cell debris. The supernatant was then centrifuged at 12,500 g for 15 min and the pellet was suspended in the mannitol solution. The tissue preparation (minus the ROSs) was then layered on a discontinuous ficoll gradient (8,16, and 20% in 0.3 M mannitol) and centifuged at 63,000 g for 1 hr in a swinging bucket rotor. The pellet at the bottom of the gradient contained mitochondria. The mitochondria were suspended in bicarbonate buffer ($NaHCO_3$, 50 mM; NaCl, 50 mM; KCl, 50 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 2 mM; $CaCl_2$, 10 μM, pH 7.4) as described by Kuo and Miki (1980).

Phosphorylation assay and polyacrylamide gel electrophoresis (PAGE)

The incubation mixture containing bicarbonate buffer and mitochondrial fraction (~0.1 mg protein), was preincubated for 2 min in a shaking water bath at 37° and the phosphorylation reaction was started by the addition of [γ-^{32}P]ATP (20 μCi, 10 μM). The system was incubated for 6 min which was determined to be in the linear time range for phosphorylation. The reaction was then stopped by adding 0.3 ml of gel electrophoresis sample buffer [60 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, 2 mM mercaptoethanol, and 0.00125% bromophenol blue] and immediately boiling for 5 min. Aliquots of the incubation mixture were subjected to PAGE on 12% gels according to the method of Laemmli (1970). The gels were dried and exposed to X-ray film to visualize the incorporation of radioactive phosphate into the various proteins. The film was developed after 1–2 days exposure at −80°C. A densitometer was used to measure the relative quantity of radioactivity in the designated phosphoprotein band (~20 K molecular weight). The densitometer measured a middle portion of the phosphoprotein band. Densities of the radioactive exposure observed in the ~20 K M_r phosphoprotein were determined to be in a linear range for the densitometer by exposing X-ray film for predetermined times with known amounts of radioactivity.

Results

Retinal depletion and functional deficits

As prelude to the determination of the retinal protein phosphorylation, our study determined the taurine depletion in the retinal tissue, the functional deficits resulting from the taurine depletion, and the ability of the retinal tissue to recover from the depletion. The time course for the decrease in the retinal taurine content of animals placed on GES is shown in Fig. 1. After one week the taurine content had decreased by approximately 50% and reached a minimum after 16 weeks. The retinal taurine contents of rats maintained on regular drinking water or on various regimens of taurine (1.5%) or GES

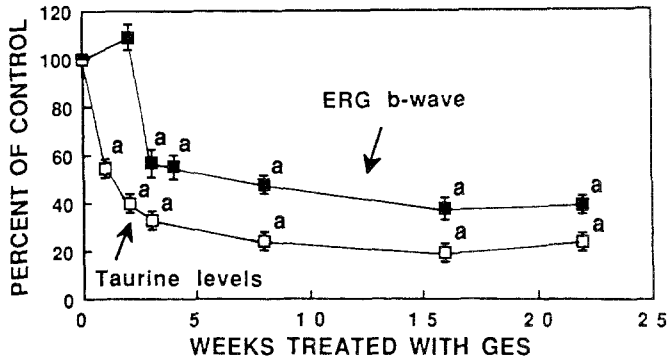

Fig. 1. ERG b-waves and taurine levels in the retinas of rats treated with GES (1½%) in the drinking water for 22 weeks. ERG measurements and determination of taurine content are described in the Materials and methods section. Data are expressed as the percent of control values (means ± SEM). Each mean for ERG measurements contain 5–8 animals. Each mean for taurine content contains 6–7 animals. Statistical differences between control values and the values obtained from animals maintained on GES for up to 22 weeks were determined by analysis of variance and the Duncan's multiple-range test (**a** $P < 0.05$). Taurine levels (□), ERG b-wave (■)

(1.5%) for 16 and 22 weeks are shown in Table 1. At each time period the taurine content was reduced by approximately 80% in animals given GES in the water.

ERG b-wave measurements performed at specific time periods on rats maintained on GES for a 22 week period are shown in Fig. 1. At two weeks GES had no effect. However, at 3 weeks the amplitude of the ERG b-wave decreased by approximately 40%. The maximum decline in the ERG b-wave amplitude was observed after 16 weeks of GES.

Retinal recovery from depletion

Animals which were placed on GES for 16 weeks and then on regular water for an additional 6 weeks increased their retinal taurine content to 76% of control value while animals placed on taurine water for the additional 6 weeks increased their taurine content to 99% of control (Table 1). The retinal taurine content in control animals given water containing taurine increased by 18%.

Recovery of the ERG b-wave after removing GES from the drinking water at 2 time points (+2 weeks and +6 weeks) is shown in Table 2. At +2 weeks after the initial 16 week treatment on GES the amplitude of the b-wave was still reduced when the animals were administered only water (44% of control) or water containing 1.5% taurine (59% of control). However, at +6 weeks the amplitude of the b-wave of the GES-treated animals which were given taurine increased to 88% of the control value and was statistically not different than the control value. The magnitude of the b-wave amplitude of animals that were given only water for an additional 6 weeks was lower than the control value (81%) but greater than animals continued on GES (39%).

Table 1. Retinal taurine content of taurine-depleted rats fed water or taurine

Treatment	16 weeks			+6 weeks		
	Taurine content	(N)	% of control	Taurine content	(N)	% of control
	nmoles/mg protein			*nmoles/mg protein*		
Control	346 ± 30[a]	(6)	100	317 ± 14[a]	(7)	100
Control/taurine	–	–	–	374 ± 4[b]	(7)	118
GES/water	–	–	–	242 ± 11[c]	(6)	76
GES/taurine	–	–	–	315 ± 10[a]	(6)	99
GES	65 ± 4[b]	(6)	19	75 ± 6[d]	(7)	24

Two groups of animals were maintained on either untreated water (*Control*) or 1.5% GES (*GES*) in the drinking water for 16 weeks. The control groups were then subdivided into two groups: one group was maintained on water (*Control*) and one group received 1.5% taurine in the drinking water (*Control/taurine*) for the next 6 weeks. The GES group was subdivided into three groups: one group received water (*GES/water*), the second group received 1.5% taurine in the drinking water (*GES/taurine*), and the third group was maintained on GES (*GES*) for the next 6 weeks. Taurine levels were determined at the end of the initial 16 week period (*16 weeks*) and after 6 weeks on the various treatments (*+6 weeks*). Data are expressed as the means ± SEM. Values in parentheses are the number of animals per group. Analysis of variance and the Duncan's multiple-range test were used to determine significant differences between the various groups of animals. Means with different superscript letters are significantly different from each other within columns ($P < 0.05$).

Effects of taurine depletion and repletion on protein phosphorylation

Phosphorylation of a specific protein present in the mitochondrial fraction of the rat retina and with an apparent molecular weight (M_r) of approximately 20K was increased in the rats treated for 6 weeks with GES. Representative autoradiograms from a 1-dimensional PAGE gel demonstrating these data are shown in Fig. 2. Densitometry measurements of the exposed film in the area of the ~20K M_r protein showed that GES treatment increased the phosphorylation of the ~20K M_r protein by 94% (Fig. 3).

After animals were partially depleted of their taurine stores by treatment with GES for 6 weeks, the GES was replaced with taurine (1.5%) in the drinking water for an additional 6 weeks to determine if the phosphorylation of the ~20K M_r protein would return to control values upon reversal of the taurine depletion. As can be seen from the representative autoradiogram and the bar graph shown in Figures 2 and 3 (right column), the magnitude of the phosphorylation of the ~20K M_r protein, under these conditions of reversing the taurine depletion, appears similar to the control value. In addition, when animals were treated only with taurine (1.5%) in the drinking water for 6 weeks, the phosphorylation of the ~20K M_r protein also was similar to the control values (middle columns). Thus, the theoretical possibility of a decrease in the phosphorylation of the ~20K M_r protein due to increased tissue

Table 2. Electroretinogram b-wave measurements in taurine-depleted rats fed water or taurine

Treatment	16 weeks			+2 weeks			+6 weeks		
	Amplitude of b-ware μV	(N)	% of control	Amplitude of b-ware μV	(N)	% of control	Amplitude of b-wave μV	(N)	% of control
Control	543 ± 31[a]	(8)	100	839 ± 61[a]	(6)	100	715 ± 26[a]	(7)	100
Control/taurine	–	–	–	786 ± 48[a]	(6)	94	651 ± 44[ab]	(7)	91
GES/water	–	–	–	370 ± 20[b]	(6)	44	577 ± 38[bc]	(7)	81
GES/taurine	–	–	–	497 ± 69[b]	(6)	59	626 ± 41[ab]	(6)	88
GES	209 ± 25[b]	(8)	38	–	–	–	280 ± 19[d]	(7)	39

Maintenance schedules of the animals on water, GES, or taurine are described in the legend to Table 1. ERG b-waves were measured at the end of the initial 16 week period (16 weeks), after 2 weeks on the various treatments (+2 weeks), and after 6 weeks on the various treatments (+6 weeks). Data are expressed as the means ± SEM. Values in parentheses are the number of animals per group. Analysis of variance and the Duncan's multiple-range test were used to determine significant differences between the various groups of animals. Means with different superscript letters are significantly different from each other within columns ($P < 0.05$).

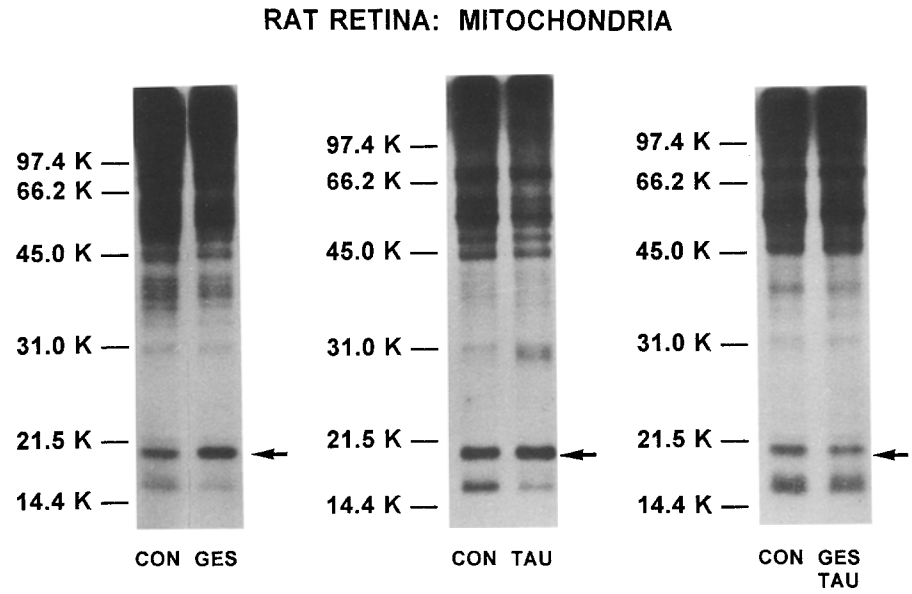

Fig. 2. Autoradiograph from a 1-dimensional (PAGE) gel of the effects of taurine depletion, taurine treatment, and reversal of taurine depletion on the phosphorylation of an ~20K M_r protein present in a mitochondrial fraction of the rat retina (representative experiment). Left column: Animals were treated for 6 weeks with GES (1.5%) in the drinking water. Middle column: Animals were treated for 6 weeks with taurine (1.5%) in the drinking water. Right column: Animals were treated for 6 weeks with GES (1.5%) followed by 6 weeks of treatment with taurine (1.5%) in the drinking water. Description of the phosphorylation assay, PAGE, autoradiography, and densitometry are described in the Materials and methods section. Marker proteins with molecular weights ranging from 14.4 to 97.4K are indicated. *CON* control; *GES* guanidinoethanesulfonic acid; *TAU* taurine. The arrow indicates the location of a protein with an approximate molecular weight of ~20K

levels of taurine was not observed. Such results suggest that endogenous taurine has already maximally inhibited the phosphorylation of the ~20K M_r protein and further loading (*in vivo*) the retina with taurine has no added effect.

Discussion

In rats, chronic taurine deficiency leads to profound, but reversible, deficits in retinal taurine stores and in the neural transmission of electrophysiological signals through the inner retina. The experiments reported herein constitute one of the longest chronic studies involved in depleting the levels of taurine in the retina of the rat with the taurine transport inhibitor, GES. The total period of these studies spanned 22 weeks. [Effects of GES and other taurine analogues in reducing the tissue levels of taurine have been reviewed by Huxtable et al. (1979) and Shaffer and Kocsis (1981)]. Previously, it had been reported by Lake et al. (1986) that taurine depletion over 15 weeks results in a deple-

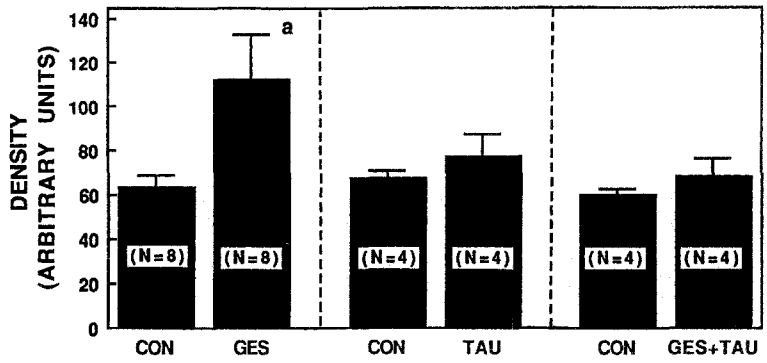

Fig. 3. Quantitative effects of taurine depletion, taurine treatment, and reversal of taurine depletion on the phosphorylation of an ~20K M_r protein present in the mitochondrial fraction of the rat retina. Left panel: The animals were treated for 6 weeks with GES (1.5%) in the drinking water. Middle panel: Animals were treated for 6 weeks with taurine (1.5%) in the drinking water. Right panel: Animals were treated for 6 weeks with GES (1.5%) followed by 6 weeks of treatment with taurine (1.5%) in the drinking water. Measurement of the phosphorylation of the ~20K molecular weight protein is described in the Materials and methods section. Data are expressed as means ± SEM in arbitrary densitometry units for 6 experiments. Statistical differences between the control (*CON*) values and the treatments were determined by the Student's t-test ($P < 0.05$)

tion of retinal taurine content by 70% with a 57–71% reduction in the amplitude of the ERG b-wave depending upon the stimulus intensity. In a subsequent study, Lake (1987) treated animals with 1.6% GES for 20 weeks and then used taurine to reverse the cardiovascular changes observed with the long-term taurine depletion. In our present studies we report that the retinal taurine content is precipitously reduced during the first 2–3 weeks of GES treatment (to 33% of control) and then continues to slowly diminish to 19 and 24% of control values at 16 and 22 weeks of GES treatment. It thus appears that the maximum achievable depletion in taurine content of the retina is 80% even after extensive, long-term treatment with a taurine-depleting agent.

In the present study, the amplitude of the ERG b-wave fell with the decrease in taurine in taurine content (Fig. 1). But it is interesting to note that the amplitude of the ERG b-wave remained constant for the first 2 weeks of GES treatment in contrast to the rapid decrease in taurine content (Fig. 1). This observation suggests that attenuation of the b-wave, a presumed physiologic consequence of taurine depletion, is not immediate but has a brief lag period. Also, we then observed that the decline in the amplitude of the ERG b-wave parallels with the decrease in the taurine content for the next 20 weeks reaching minimum values of 38 and 39% of control values at 16 and 22 weeks. Lake (1989) reported that though the decrease in the taurine level of the retina plateaued after 6 weeks of GES treatment, the amplitude of the ERG b-wave continued to decrease after 6 weeks of GES treatment and reached a minimum level (30% of control) after 14 weeks of GES treatment. In our laboratory, it appears that a 60% decrease in the amplitude of the b-

wave is the maximum achievable effect after chronic, long-term treatment with GES. The greater amplitude decrement in the studies of Lake (1989) may be due to light exposure during repeated 40-minute testing sessions (personal communication). Exposure to bright flashes may accelerate/potentiate the ERG losses and may increase the chances of structural damage.

When the prolonged GES treatment is discontinued, we found, as Cocker and Lake (1987) had observed after 4 weeks of treatment, that both the retinal taurine content and the ERG b-wave significantly increased (Tables 1 and 2). When fed taurine in the drinking water, the recovery to control values is virtually complete. The recovery of the ERG b-wave is particularly interesting because the b-wave depends on the transmission of sizable, if not normal, photoreceptor signals to the inner retinal neurons, on the ability of depolarizing bipolar cells to generate electrical currents, and also on the integrity of Muller cells which extend the full thickness of the retina. The recovery implies that cell death or permanent function deficits were not obvious even after 16 weeks of GES treatment.

The main novel finding of the present study is a substantive increase in phosphorylation of a specific retinal protein (Figs. 2–3). The present finding is not likely attributable to some aberrant effect associated with massive cell death because, as we had carefully examined even after chronic treatment, the effects of the GES treatment in the rats are reversible. The more likely explanation is that taurine serves as an endogenous modifier of the protein, an hypothesis supported by previous *in vitro* experiments showing that the phosphorylation of this ~20K M_r protein is inhibited by taurine with an IC_{50} (concentration required for 50% inhibition) of 34.2 ± 2.1 mM (Lombardini, 1993). Accordingly, one would expect that in the partially taurine-depleted rats *in vivo* phosphorylation of the ~20K M_r protein would be *increased* since the concentration of the endogenous inhibitor, taurine, was decreased. Thus, it was anticipated that the *in vitro* phosphorylation with [γ-^{32}P]ATP of the serine(s) and threonine(s) amino acid residues (Lombardini, 1993) in the ~20K M_r protein would be *decreased* due to the hydroxyl moieties reacting with non-radioactive ATP when the animal was alive. But, we observed the opposite effect, i.e., phosphorylation with radioactive ATP was increased in the GES-treated animals. Thus, it appears when retinal levels of taurine are reduced in the whole animal, there may be enhanced phosphorylation along with enhanced dephosphorylation, i.e., greater turnover of phosphate groups than in the normal animal. This situation would allow for increased incorporation of radioactive phosphate into the ~20K M_r protein as was observed in the GES-treated rats.

Alternatively, other explanations may be possible and have not been ruled out. First, it is well known that the tissues of animals treated with GES take up GES in place of taurine (Lake et al., 1987; Huxtable et al., 1979). It has also been previously reported that 20 mM GES inhibits Na/K ATPase activity by 60% (Lombardini, 1985). Thus, it is possible that the GES in the mitochondrial fraction of the retina inhibits ATPase activity thereby decreasing the use of the radioactive ATP and subsequently shifting more of the

labelled ATP into phosphorylation. This increased usage of the [γ-^{32}P]ATP for phosphorylation would appear to be an increase in kinase activity.

Second, it has been reported by Lake and colleagues (Lake and Malik, 1987; Lake, 1989) that GES treatment results in inner retinal shrinkage. Thus GES treatment could alter the composition of the proteins in the retinal extract and thus change the degree of *in vitro* phosphorylation.

The autoradiograms (Fig. 2) show that there are both inhibitory and stimulatory effects due to taurine depletion (GES treatment) and taurine treatment on the phosphorylation of proteins other than the ~20K M_r protein found within the retinal mitochondrial fraction. However, while the effects of either GES or taurine treatment of the animals on the phosphorylation of these additional proteins are of potential interest, these phosphoproteins have not been investigated for the following reasons: (1) some of the phosphoproteins are in regions of the gel in which separation by polyacrylamide gel electrophoresis is less defined, and (2) their phosphorylation reaction is not consistent in all mitochondrial preparations as viewed by autoradiography.

In conclusion, results from these studies show that a substantive increase in phosphorylation of an ~20K M_r protein present in the mitochondrial fraction of the rat retina occurs when the taurine content of the retina was decreased and the amplitude of the ERG b-wave was reduced. It is not entirely clear how or whether these observations are related, but if they are, it may be that taurine regulates the phosphorylation of certain, specific proteins that are essential to the retinal light response but not to the structural integrity of the neurons. Accordingly, future studies to determine the function of the ~20K M_r protein in retinal tissue of normal and taurine-depleted animals are warranted.

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