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Combined effects of guanidinoethanesulfonate, a depletor of tissue taurine levels, and isoproterenol or methoxamine on rat tissues

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Taurine, a ubiquitous amino acid constituent of mammalian tissues, has been implicated as having a physiologic role in cardiac function. A definitive mechanism for taurine in cardiac tissue, however, has thus far eluded investigators. It has been proposed that taurine is an antiarrhythmic agent capable of reversing drug-induced electrical abnormalities [1, 2]. Various studies have also suggested that taurine might alter cardiac function by modulating calcium utilization [3–5]. Guidotti *et al.* [3] have demonstrated that taurine increases the positive inotropic effects of strophanthin in the perfused heart. Likewise, Dietrich and Diacono [4] and Iwata and Fujimoto [5] have also reported that taurine potentiates the inotropic effect of ouabain. Moreover, it has been demonstrated that taurine increases calcium binding rate and calcium content of the sarcoplasmic reticulum [6]. Evidence of a function for taurine in cardiac tissue has been reported by Chovan *et al.* [7] who suggested that low-affinity taurine binding sites appear to regulate calcium levels in the cardiac sarcolemma.

Huxtable *et al.* [8] have postulated that part of the difficulty in defining a mechanism for taurine is the inability of the investigator to modify the *in vivo* taurine content of cardiac tissue. In addressing this problem, they [8] demonstrated that the rat can be partially depleted of its tissue stores of taurine by the addition of 1.0% guanidinoethanesulfonate (GES) to the drinking water. GES, a structural analogue of taurine, depletes tissue taurine content by inhibiting taurine transport. However, while the extent of taurine depletion was considerable, for example after 4 weeks of treatment cardiac tissue contained only 20 per cent of control taurine levels, liver, 24 per cent and cerebellum, 33 per cent, additional treatment with GES did not deplete taurine content below these values [8]. In addition, results from our laboratory [9] have demonstrated that isoproterenol (ISO) and methoxamine (MOX), both sympathomimetic agents, diminish the taurine content of cardiac tissue by 30–40 per cent and enhance the concentrations of taurine in blood by 70–100 per cent.

In the present communication, the combined effects of GES and ISO or GES and MOX on rat tissues were investigated.

Male Sprague–Dawley rats weighing 250–290 g were used in all experiments. The animals had free access to water and food and were fed Ralston Purina Rodent Laboratory Chow No. 5001. Experimental animals were maintained on drinking water containing 1.5% GES for 35 days. GES was synthesized and characterized as described by Huxtable *et al.* [8].

DL-Isoproterenol (Sigma Chemical Co., St. Louis, MO) and methoxamine hydrochloride (supplied by Mr. C. A. Parish, Jr., Burroughs Wellcome & Company, Research Triangle Park, NC) were dissolved in 0.9% NaCl and administered as subcutaneous injections. Control animals received injections of saline. Seven hours after ISO (80 mg/kg), MOX (20 mg/kg), or saline administration, the animals were anesthetized with ether, and 5–7 ml of blood was withdrawn from the inferior vena cava and placed in vacutainer tubes containing EDTA. The blood was deproteinized with an equal volume of 5% perchloric acid and centrifuged for 10 min at 12,000 *g*. Tissues were rapidly removed, rinsed in cold saline, and blotted to remove excess moisture. The tissues were then weighed and homogenized in 2 vol. of 2% perchloric acid with a Polytron homogenizer. The homogenates were centrifuged as above.

Taurine content was determined in the tissue supernatant solutions on an amino acid analyzer (Beckman model 121) utilizing W-1 resin (Beckman-Instruments, Inc., Fullerton, CA) and sodium citrate buffer (0.2 N, pH 2.4). GES and other naturally occurring guanidino compounds were analyzed according to the colorimetric procedure described by Huxtable *et al.* [8]. The chromatographic steps utilized to separate GES from the naturally occurring guanidino compounds were omitted when the cardiac tissue levels of naturally occurring guanidino compounds were to be quantitated. Dry/wet tissue-weight ratios were calculated in all experiments, to express the analytical results on a dry weight basis.

The effects of treatment with GES alone or in combination with ISO or MOX on various rat tissues are shown in Figs. 1 and 2. In agreement with Huxtable *et al.* [8], it was observed that GES depleted the taurine contents of heart and spleen to 25 and 28 per cent, respectively, of control values. Kidney and liver tissues were depleted of their taurine contents to a greater degree than reported by Huxtable *et al.* [8], but different conditions were utilized. Taurine contents of blood and brain were reduced to 32 and 39 per cent, respectively, of control values. Administration of ISO to GES pretreated rats further depleted the content of taurine in cardiac tissue to 16.4 per cent of control value. Similar results were obtained after administration of MOX, that is, a further depletion of cardiac tissue taurine content occurred but to a lesser extent than after ISO treatment.

Administration of ISO or MOX to animals treated previously with GES increased the concentrations of taurine in the blood by 21 per cent ($P < 0.02$) and 84 per cent ($P < 0.005$) respectively; no changes in taurine levels were observed after treatment with ISO or MOX in the liver, spleen, or brain (Figs. 1 and 2). However, a small but significant increase ($P < 0.01$) in kidney taurine levels was observed after MOX administration when compared to taurine levels of animals treated with GES alone. ISO administration had no effect on the kidney taurine levels of GES-treated animals.

The content of guanidino compounds in cardiac tissue is shown in Fig. 3. Control hearts contained a total of 51.6 ± 4.4 μmoles/g dry wt of naturally occurring guanidino compounds. After administration of 1.5% GES in the drinking water for 35 days, the content of total guanidino compounds was 141.3 ± 4.4 μmoles/g dry wt. GES accounted for 80 per cent (113.5 ± 1.9 μmoles/g dry wt) of the total, while the other naturally occurring guanidino compounds accounted for the remaining 20 per cent (27.8 ± 3.3 μmoles/g dry wt).

It was reported previously that the contents of most of the amino acid constituents of cardiac tissue were not altered 7 hr after administration of either ISO or MOX [9]. The amino acids which were exceptions to this general statement were taurine, threonine, glutamine plus asparagine, and glutamate which had decreased in content, and alanine and aspartate which had increased; the arginine content of cardiac tissue did not change after ISO administration. In the present study both ISO and MOX reduced the cardiac tissue content of total guanidino compounds

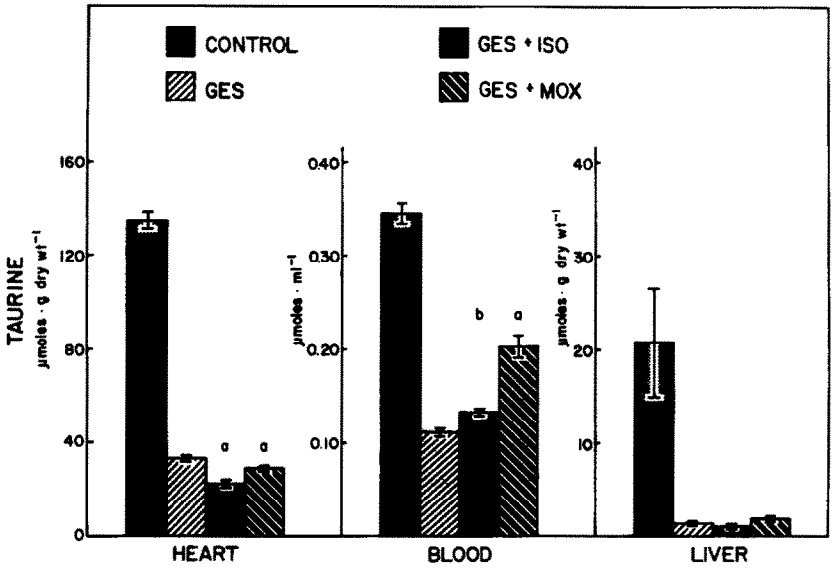

Fig. 1. Effects of ISO or MOX on tissue levels of taurine in animals chronically treated with GES. Animals were maintained on 1.5% GES in the drinking water for 35 days. Tissues were analyzed for taurine content 7 hr after administration of ISO (80 mg/kg), MOX (20 mg/kg), or saline. Taurine levels are expressed as μmoles/g dry wt for tissues and μmoles/ml for blood. Six animals were used in each treatment group (means ± S.E.). The values for P were calculated by the Wilcoxon 2-sample test [(a) $P < 0.005$; and (b) $P < 0.02$] for comparison between GES and GES + ISO or GES + MOX data groups.

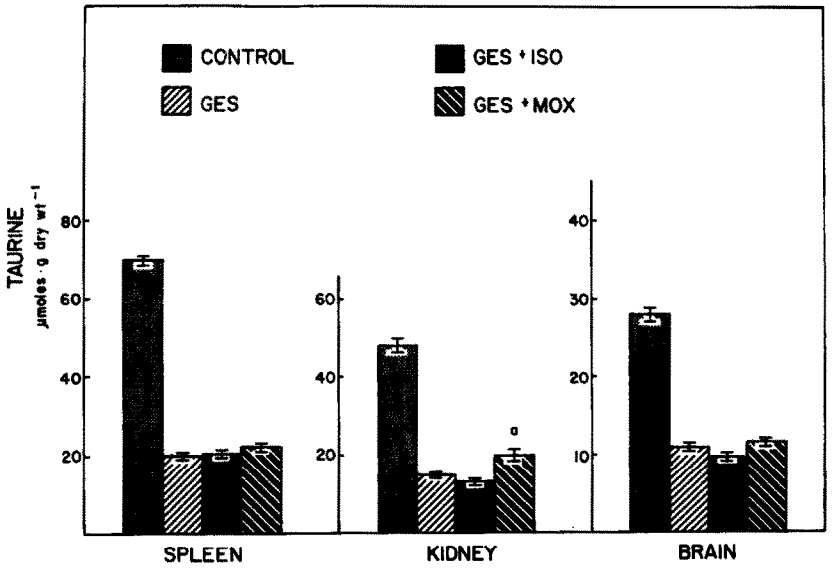

Fig. 2. Effects of ISO or MOX on tissue levels of taurine in animals chronically treated with GES. Conditions of the experiments are stated in the legend to Fig. 1. (a) The GES + MOX data group was significantly different from the GES group, $P < 0.01$.

after GES treatment by 9.2 and 13.5 per cent respectively (GES + ISO, $P < 0.002$; GES + MOX, $P < 0.005$) (Fig. 3). When the total content of guanidino compounds was divided into either the content of GES or of other naturally occurring guanidino compounds, however, it was observed that both ISO and MOX caused a decrease of only the GES contents of cardiac tissue (GES + ISO, $P < 0.01$; GES + MOX, $P < 0.005$) while not affecting the naturally occurring guanidino compounds. GES treatment reduced the cardiac content of the other guanidino compounds when compared to the control values ($P < 0.005$).

The combined treatments, of GES in drinking water and administration of ISO or MOX, produced a 78–84 per cent depletion of taurine in cardiac tissue. After recovery from the adverse effects of ISO or MOX, however, the apparent well-being of the animals was not markedly different from

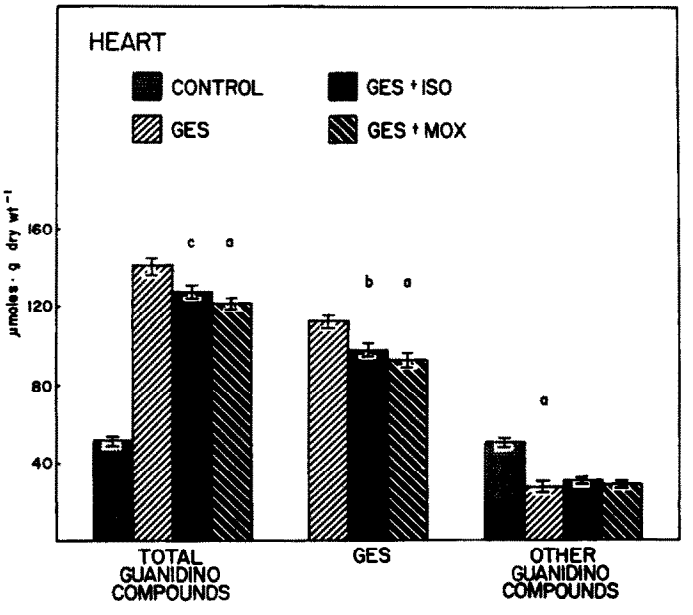

Fig. 3. Effects of ISO or MOX on cardiac tissue levels of guanidino compounds in animals chronically treated with GES. Animals were maintained on 1.5% GES in the drinking water for 35 days. Tissues were directly analyzed for total guanidino compounds and GES by the procedure of Huxtable *et al.* [8] 7 hr after administration of ISO (80 mg/kg), MOX (20 mg/kg), or saline. Tissue levels of the indicated compounds are expressed as μmoles/g dry wt. Six animals were used in each treatment group (means ± S.E.). The values for P were calculated by the Wilcoxon 2-sample test [(a) $P < 0.005$; (b) $P < 0.01$; (c) $P < 0.02$].

that of control animals or animals that had received only GES. Once again the unanswered query must be considered: "What is the function of taurine in the heart?". Huxtable *et al.* [8] suggested three possible conclusions from their experiments utilizing GES: (1) that taurine is nonfunctional; (2) that only a small residual fraction of taurine is necessary for proper physiological status of the animal; and (3) that GES, which replaces taurine in depleted tissue, is a taurine agonist. Most investigators with an interest in taurine would rather not consider point 1. Point 2, that only a small fraction of the taurine pool in a tissue is important, has some merit when one considers that retinal damage in the cat can be demonstrated only when the animal is severely depleted of its taurine stores [10]. Finally, point 3, that GES is a taurine agonist, is also a distinct possibility. Huxtable *et al.* [8], however, suggested that GES, at least in the brain, is a taurine antagonist because GES promotes seizures in seizure-susceptible rats whereas taurine possesses anticonvulsant actions. In contrast, the anticonvulsant ability of taurine in cats with penicillin-focus epilepsy has been questioned [11] and, thus, taurine and GES may indeed have the same pharmacological actions. In the experiments reported herein, one would not have expected to see any abnormality in the animals if taurine was simply being replaced by a functionally similar compound. If, on the other hand, GES was a taurine antagonist, then one would have expected to observe some form of abnormality in animals which transported 113 μmoles/g dry wt of GES into their cardiac tissue.

The mechanisms by which both an α-agonist, MOX, and a β-agonist, ISO, decrease taurine content in cardiac tissue while possessing opposing pharmacological properties are perhaps due to the fact that extremely high, acute doses of both these sympathomimetic agents produce tissue necrosis as a consequence of ischemia [12–14]. High doses of ISO reduce coronary perfusion pressures [15], whereas the myolytic lesions of cardiac tissue evoked by MOX are caused by alterations in the metabolism of the myocardial fibers in addition to localized or generalized hemodynamic changes [16]. On the contrary, it has been reported that ISO, administered chronically, produces an increase in the total taurine content of the hypertrophying heart [17]. It is unclear why, in our experiments, a reduction in taurine content was observed, whereas in the report of Chubb and Huxtable [17] an increase in cardiac tissue levels of taurine was documented. The answer may lie in the interval between the times when the animals were killed and the cardiac tissue was analyzed for taurine content. In the experiments reported herein, the animals were killed 7 hr after drug administration. In previous experiments from our laboratory, it was noted that by 48 hr post-ISO administration or by 72 hr post-MOX administration the taurine content of the cardiac tissue returned to control values. In the experiments reported by Chubb and Huxtable [17], in which ISO was given chronically, the animals were not killed until 5 to 18 days after ISO administration. Thus, sympathomimetic agents may produce a biphasic response in taurine fluxes within cardiac tissues.

ISO in a dose of 4×10^{-7} M enhances taurine influx in perfused hearts, presumably by a β-adrenergically activated cyclic AMP-mediated mechanism [18, 19]. Interestingly, Wheler and Klein [20, 21] have shown that both cyclic AMP and norepinephrine cause a release of taurine from pinealocytes, again presumably acting through β-adrenergic mechanisms. The contradiction between the two observations that a β-adrenergic mechanism produces both an influx of taurine into cardiac tissue and an efflux of taurine out of the pineal gland has not been resolved.

In conclusion, it was found that: (1) depletion of taurine levels in cardiac tissue by GES can be enhanced by the administration of high doses of ISO or MOX, and (2) cardiac tissue levels of the naturally occurring guanidino compounds are not affected by the administration of ISO or MOX. The data suggest that either cardiac tissue contains an excess of taurine with only a small fraction of the total content being functional or that GES behaves as a taurine agonist.

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