Glycerol-induced fluid shifts attenuate the vestibulosympathetic reflex in humans

Damian J. Dyckman, Charity L. Sauder, and Chester A. Ray

Penn State Heart and Vascular Institute, Department of Cellular and Molecular Physiology, General Clinical Research Center, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Submitted 22 November 2010; accepted in final form 20 December 2010

Dyckman DJ, Sauder CL, Ray CA. Glycerol-induced fluid shifts attenuate the vestibulosympathetic reflex in humans. Am J Physiol Regul Integr Comp Physiol 2011; 300:R630–R634. First published December 22, 2010; doi:10.1152/ajpregu.00767.2010.—The glycerol dehydration test (GDT) has been used to test for the presence of Ménière’s disease and elicits acute alterations in vestibular reflexes in both normal and pathological states. Activation of the vestibulosympathetic reflex (VSR) increases muscle sympathetic nerve activity (MSNA) and peripheral vascular resistance. We hypothesized that the GDT would attenuate the VSR through fluid shifts of the inner ear. Sixteen male subjects (26 ± 1 yr) were randomly assigned to be administered either glycerol mixed with cranberry juice (97 ± 3 ml glycerol + equal portion of cranberry juice; n = 9) or a placebo control (water + cranberry juice [100 ml each]; n = 7). Subjects in both groups performed head-down rotation (HDR), which engages the VSR, before and after administration of either the glycerol or placebo. MSNA (microneurography), arterial blood pressure, and leg blood flow (venous occlusion plethysmography) were measured during HDR. Before glycerol administration, HDR significantly increased MSNA burst frequency (Δ8 ± 1 bursts/min; P < 0.01) and total activity (Δ77 ± 18%; P < 0.01) and decreased calf vascular conductance (−Δ20 ± 3%; P < 0.01). However, HDR performed postadministration of glycerol resulted in an attenuated MSNA increase (Δ3 ± 1 bursts/min, Δ22 ± 3% total activity) and decrease in calf vascular conductance (−Δ7 ± 4%). HDR significantly increased MSNA burst frequency (Δ5 ± 1 and Δ5 ± 2 bursts/min) and total activity (Δ58 ± 13% and Δ52 ± 18%) in the placebo group before and after placebo, respectively (P < 0.01). Likewise, decreases in calf vascular conductance during HDR before and after placebo were not different (−Δ13 ± 4% and −Δ14 ± 2%, respectively; P > 0.01). These results suggest that fluid shifts of the inner ear via glycerol dehydration attenuate the VSR. These data provide support that inner ear fluid dynamics can have a significant impact on blood pressure regulation via the VSR in humans.

Orthostatic intolerance is a significant problem experienced in the elderly (23) and in approximately two-thirds (5) of all astronauts upon returning to Earth. The mechanism of this problem is not clearly understood. In astronauts, many factors contribute to this problem, ranging from hypovolemia, changes in baroreceptor activation, sympathetic withdrawal, and an inability to increase peripheral vascular resistance (5, 7, 9, 11, 16, 21, 41). The most severe cases of orthostatic intolerance result from an inability to augment total peripheral vascular resistance (5, 11, 22, 41). In addition, space motion sickness is experienced by up to 80% of astronauts (31). It has been hypothesized that motion sickness develops from fluid shifts that alter intracranial, cerebrospinal fluid, or inner ear fluid pressures, altering properties of the vestibular receptors (14, 33). Data from animals and humans have demonstrated the presence of a vestibular mediated sympathetic reflex that can contribute to autonomic control of sympathetic nerve activity, vascular resistance, and blood pressure (8, 15, 19, 32, 42, 43). Using head-down rotation (HDR) as a model to activate the vestibular otolith organs, our laboratory has reported increased muscle sympathetic nerve activity (MSNA) and peripheral vasoconstriction during this maneuver in humans (15, 26–29, 32). Other studies in humans have reinforced this concept of the vestibulosympathetic reflex (VSR) (2, 10, 19, 39).

Ménière’s disease serves as a model to understand inner ear fluid dynamics (1). Patients with Ménière’s disease have excessive fluid accumulation within the membranous labyrinth of the inner ear (1, 35). This results in vestibular symptoms of vertigo, tinnitus, and hearing loss (1, 35). The medical management of the disease includes the use of diuretics, which decreases the fluid volume within the body and in the inner ear (17, 34, 35). Classically, the glycerol dehydration test (GDT) has been used to test for the presence of Ménière’s disease (12, 24, 25). It has been demonstrated that glycerol can reduce intraocular pressure by decreasing cerebrospinal fluid volume (3), decreasing intracranial pressure (4), and causing fluid shifts within the inner ear in humans (24, 35). Glycerol can also modify the electrolyte concentration of both the perilymph and endolymph within the inner ear (18, 24, 36). Currently, no studies have investigated the direct effect of fluid shifts of the inner ear on the VSR in humans.

The primary goal of this study was to test whether acute fluid shifts within the vestibular apparatus affect the VSR. We hypothesized acute glycerol dehydration would decrease the sensitivity of the VSR and would lead to reduced peripheral vasoconstriction. These findings might have important clinical implications. Acute fluid shifts within the vestibular apparatus might contribute to the attenuated VSR and increased orthostatic hypotension in the elderly. This might also provide insight into the increased prevalence of orthostatic intolerance in astronauts following spaceflight.

METHODS

Subjects. Sixteen young healthy male volunteers (age: 26 ± 1 years; height: 178.5 ± 2.0 cm; weight: 78.8 ± 3.3 kg) participated in this study. All subjects were nonsmokers, nonobese, normotensive, and not taking any medications that could influence the results of the study. The Institutional Review Board of the Pennsylvania State University College of Medicine approved the experiment, and written informed consent was obtained from all subjects before testing.

Experimental design. Subjects were randomly assigned to perform the experimental protocol before and after either glycerol mixed with cranberry juice (n = 9) or placebo (water + cranberry juice; n = 7).
HDR was performed before and after administration of either the glycerol or placebo. A testing day consisted of a control HDR trial and cold pressor test followed by drug administration (placebo or glycerol), waiting an hour, and then a second HDR trial and cold pressor test. During the 1-h break between the two HDR trials, subjects were disconnected from all instrumentation and permitted to move around. Forty-five minutes after glycerol or placebo ingestion, subjects were instrumented, and the nerve site was established at the same location of the peroneal nerve as the first trial. The same site was used because recording during the first session was short (<20 min) and allowed for rapid establishment of a recording site for the second trial. All subjects started the second trial of HDR and a cold pressor test 60 min after glycerol or placebo ingestion. Urine was collected during this time. At the start of each HDR trial, a blood sample was obtained for hematocrit determination, and an estimated change in plasma volume was calculated using van Beaumont’s equation (38). All experiments were performed in a dimly lit, quiet laboratory maintained between 21 and 23°C.

Vestibular activation. Vestibular otolith activation was tested before and after drug administration by the subjects performing HDR in the prone position, as previously described (32). This maneuver engages the otolith organs, but not the semicircular canals when the head becomes stationary. Subjects were placed in the prone position, instrumented for the study (BP, heart rate, venous occlusion plethysmography), and an appropriate MSNA recording site was obtained. After a 3-min baseline period with the head in the baseline chin-up neck extended position, the chin support was removed and the head was passively rotated to the point of maximal rotation. This position was maintained for 3 min followed by the subject’s head being passively returned to the baseline chin-up neck-extended position for 3 min of recovery.

A cold pressor test was performed following the HDR trial. The cold pressor test consisted of a 2-min baseline period followed by placing the subject’s hand in ice water up to the wrist for 2 min. During HDR and the cold pressor test, arterial blood pressure, heart rate, MSNA, and venous occlusion plethysmography of the contralateral leg were continuously recorded.

Drug administration. After the initial HDR trial was performed, subjects consumed either 100 ml water mixed with 100 ml cranberry juice (200 ml total), which served as a placebo control, or 1.5 g/kg glycerol (on average 97 ± 3 ml) mixed with an equal portion of cranberry juice. The amount of water consumed was comparable to the calculated amount of glycerol subjects would have taken. Subjects were required to wait 1 h after consumption to collect urine and for the maximal physiological effect of the glycerol before beginning posttesting.

Measurements. Microneurography was used to assess MSNA as previously described (32). Briefly, multifiber recordings of MSNA were obtained from a tungsten microelectrode inserted in the peroneal nerve behind the knee. A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. Previously identified criteria for an adequate MSNA signal were applied to ensure proper recording (37). The nerve signal was amplified (20,000–40,000 times), fed through a band-pass filter with a bandwidth of 700–2,000 Hz, integrated using a 0.1-s time constant (University of Iowa Bioengineering, Iowa City, IA), and recorded digitally (16SP Powerlab; ADInstruments, New Castle, Australia). The mean voltage neurogram was routed to a computer screen and a loudspeaker for monitoring during the study. Sympathetic recordings that demonstrated possible electrode site shifts, or electromyographic artifact during experimental intervention were excluded from analysis.

Venous occlusion plethysmography (Hokanson EC 4 plethysmograph, D. E. Hokanson, Bellevue, WA) was used to assess calf blood flow of the contralateral leg, as previously described (15). Briefly, mercury-in-Silastic strain gauges were placed around the maximal circumferences of the calf. An ankle cuff was inflated to 220 mmHg to arrest circulation in the foot while a thigh cuff was inflated to 50 mmHg every 15 s. Calf vascular conductance was calculated as the ratio of calf blood flow to mean arterial blood pressure. Heart rate was derived from an electrocardiogram. Arterial blood pressure was measured continuously by finger photoplethysmography (Finometer, FMS, Amsterdam, The Netherlands) during each trial.

Data analysis. All data were digitally recorded at 100 Hz for later off-line analysis. The investigator analyzing the data was blinded to the subjects’ identity and to which drug was received. MSNA was expressed as burst frequency (per minute) and total activity (sum of area under individual bursts per minute, expressed in arbitrary units). Neurograms were normalized using the highest burst recorded during baseline set to 1,000 and a period without burst activity set to zero. This procedure was repeated for each new MSNA site obtained. Sympathetic bursts were identified from the mean voltage neurogram and the sum of the area under each burst, expressed in arbitrary units (a.u.), was assessed by a computer program (Chart 5; ADInstruments).

Statistics. To identify possible differences between the predrug and postdrug administration responses to HDR, a two-within (drug × HDR), one-between (group) repeated-measures ANOVA was used. When the interaction was significant, a test for simple effects was used to identify whether there were differences between time points (20). MSNA comparisons were made between the average resting baseline (baseline) activity and during the first minute of HDR. The cold pressor test data were analyzed using the average resting data (baseline) and the second minute of the cold pressor test. An unpaired t-test was used to identify differences in plasma volume and urine volume across the two conditions. A significance level of P < 0.05 was used for all tests. Values are presented as means ± SE.

Table 1. Drug administration volume alterations and hemodynamic responses to HDR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume lost</td>
<td>282 ± 92 ml</td>
<td>506 ± 65 ml</td>
</tr>
<tr>
<td>Plasma volume lost</td>
<td>−Δ5.0 ± 2.4%</td>
<td>−Δ2.7 ± 2.2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretest</th>
<th>Posttest</th>
<th>Pretest</th>
<th>Posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>79 ± 1</td>
<td>85 ± 3*</td>
<td>91 ± 2</td>
<td>94 ± 2*</td>
</tr>
<tr>
<td>HDR</td>
<td>79 ± 2</td>
<td>83 ± 3*</td>
<td>91 ± 2</td>
<td>93 ± 3*</td>
</tr>
<tr>
<td>Δ (BL vs. HDR)</td>
<td>Δ0 ± 2</td>
<td>Δ2 ± 1*</td>
<td>Δ0 ± 1</td>
<td>Δ0 ± 1</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>65 ± 3</td>
<td>63 ± 3*</td>
<td>60 ± 4</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>Δ (BL vs. HDR)</td>
<td>Δ0 ± 1</td>
<td>Δ1 ± 1</td>
<td>Δ1 ± 1</td>
<td>Δ0 ± 1</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 7 for placebo and n = 9 for glycerol. *Significantly different from pretest; P < 0.05. BL, baseline; HDR, head-down rotation; MAP, mean arterial pressure; bpm, beats per minute.
RESULTS

Baseline hemodynamic measurements and responses to HDR for both trials and each group are presented in Table 1. HDR did not significantly change MAP or HR at either pretesting or posttesting in both the placebo and glycerol groups. Percent change in plasma volume from pretesting to posttesting was $-\Delta 5.0 \pm 2.4\%$ and $-\Delta 2.7 \pm 2.2\%$, for placebo and glycerol groups, respectively ($P = 0.51$, between placebo and glycerol). Urine volume collected post-drug intervention was $282 \pm 92$ ml and $506 \pm 65$ ml, for placebo and glycerol, respectively ($P = 0.06$).

**Glycerol group.** HDR significantly increased MSNA burst frequency ($15 \pm 2$ to $23 \pm 2$ bursts/min, $\Delta 8 \pm 1$ bursts/min, $P < 0.001$) and total activity ($\Delta 77 \pm 18\%$, $P < 0.001$) during pretesting in the glycerol group, which was comparable to the placebo group (Fig. 1). Following glycerol administration, MSNA at baseline was increased ($\Delta 5 \pm 2$ bursts/min, $P < 0.05$; Fig. 1); however, the change in burst frequency and total activity during HDR was significantly attenuated after glycerol consumption compared with pretesting ($20 \pm 2$ to $23 \pm 2$ bursts/min, $\Delta 3 \pm 1$, $P < 0.05$; total activity $\Delta 22 \pm 3\%$; Fig. 1). Calf vascular conductance significantly decreased during HDR before glycerol administration ($-\Delta 20 \pm 3\%$, $P < 0.001$). However, reduction in calf vascular conductance from baseline was significantly attenuated following glycerol consumption ($-\Delta 7 \pm 4\%$, $P < 0.05$) (Fig. 2). The cold pressor test increased MSNA burst frequency ($\Delta 19 \pm 4$ and $\Delta 15 \pm 3$, $P < 0.001$) and total activity ($\Delta 360 \pm 107\%$ and $\Delta 260 \pm 89\%$, $P < 0.001$) during both the preglycerol and postglycerol trials, respectively. These results were not significantly different from each other because of the large variability ($P = 0.13$).

**Placebo group.** In the placebo group, HDR significantly increased MSNA burst frequency ($15 \pm 3$ to $20 \pm 3$ bursts/min, $\Delta 5 \pm 1$ bursts/min, $P < 0.001$) and total activity ($\Delta 58 \pm 13\%$, $P < 0.001$) during pretesting (Fig. 1). Following placebo administration, MSNA responses to HDR were comparable ($13 \pm 1$ to $18 \pm 1$, $\Delta 5 \pm 2$ bursts/min and total activity $\Delta 52 \pm 18\%$; Fig. 1). Calf vascular conductance significantly decreased during HDR during pre ($-\Delta 13 \pm 4\%$, $P < 0.001$) and posttesting ($-\Delta 14 \pm 2\%$, $P < 0.001$; Fig. 2). Calf vascular conductance responses to HDR did not differ between trials. The cold pressor test significantly increased MSNA burst frequency ($\Delta 9 \pm 3$ and $\Delta 14 \pm 2$, $P < 0.001$) and total activity ($\Delta 120 \pm 43\%$ and $\Delta 185 \pm 34\%$, $P < 0.001$) during both pretesting and posttesting, respectively. These results were not significantly different from each other.

DISCUSSION

The major finding of this study is that the VSR is attenuated after consuming glycerol. Glycerol elicited a decreased MSNA response and reduced peripheral vasoconstriction to HDR. These data provide evidence that fluid shifts of the inner ear can contribute to alterations of the VSR in humans.
Oral glycerol has the ability to cause fluid shifts in the body (3), a decrease in intracranial and intraocular pressure (4), and fluid shifts within the inner ear (13, 24, 35). The GDT has been used extensively to test for the presence of Ménière’s disease and for symptomatic relief of the disease (25). Gosepath et al. (13) demonstrated a decrease in intracochlear pressure in both Ménière’s patients and normal subjects after oral glycerol administration, thereby demonstrating glycerol can affect healthy normal subjects. In the current study, every subject experienced a headache while standing after consuming glycerol for approximately 3 h posttesting, possibly indicating that a fluid shift from the intracranial space had taken place. Glycerol is an osmotic diuretic that produces diuresis by shifting water from the intracellular compartment into the extracellular vasculature, thereby producing extracellular volume expansion, which, in turn, increases glomerular filtration rate and diuresis (3). Our results demonstrate that plasma volume decreased less, while urine output was increased after glycerol administration compared with placebo.

The exact mechanism(s) for the effect of glycerol on the VSR is unknown. We hypothesize that glycerol alters the fluid composition of the vestibular apparatus and impairs its ability to activate vestibular mediated reflexes. Animal studies examining the effect of glycerol on the vestibular apparatus have demonstrated a change in the electrolyte concentrations within the perilymph and endolymph in both the guinea pig (18, 36) and the rat (24). These changes in perilymph and endolymph are rapid, have their maximal effect at 60 to 90 min, and last for several hours (18, 24, 36). Morrison et al. (24) stated that these changes in concentrations occur by an osmotic movement of water out of the inner ear spaces in normal rats. In addition, Cohen et al. (6) demonstrated the main action of glycerol was osmotic reduction of inner ear pressure in guinea pigs. Therefore, these studies would suggest that an osmotic dehydration is occurring within the inner ear as a result of glycerol.

It could be suggested that an elevated baseline MSNA observed after glycerol consumption could be responsible for a false attenuation of the VSR. However, this effect is unlikely. The ability to increase MSNA despite an elevated resting baseline is demonstrated by the cold pressor test. Moreover, during lower body negative pressure, a condition in which baroreceptor unloading increased MSNA, HDR was able to increase MSNA to the same extent as during the normal condition (26). Therefore, elevated levels of MSNA at baseline after glycerol consumption is unlikely to be the cause of the attenuation of the VSR.

It is unlikely that other cardiovascular reflexes would be responsible for the attenuation of MSNA during HDR after glycerol. HDR was performed in the prone position, thus making changes in blood volume distribution unlikely. Additionally, there were no changes in blood pressure responses with HDR; therefore, it is unlikely the arterial baroreflexes inhibited the increase in MSNA by the VSR. Our previous studies have demonstrated that HDR specifically engages the VSR without input from other reflexes (e.g., neck afferents, baroreflexes, central command, and cerebral pressure) (27, 32).

The reduction in MSNA following glycerol was associated with a concomitant reduced calf vasoconstriction. This observation demonstrates that changes in the VSR have functional physiological significance on peripheral vascular resistance.

The reduction in calf vasoconstriction may contribute to reduced orthostatic tolerance (5).

Perspectives and Significance

Our results might have clinical implications. The VSR is attenuated in the elderly and possibly contributes to an increased incidence of orthostatic intolerance in this population (30). The mechanism for this response could be an alteration of the fluid regulation within the vestibular apparatus. Brain atrophy occurs with increasing age and increases the amount of intracranial cerebrospinal fluid volume, which could alter the fluid regulation within the inner ear (40). In addition, this study provides support for the concept that the VSR contributes to the development of postspaceflight orthostatic intolerance experienced by astronauts upon return to Earth. Although the fluid shift theory has been largely discounted in the development of space motion sickness due to an inability to reproduce the symptoms in a bed rest model (14, 33), this theory does not preclude the possibility that the overall hypovolemia of the astronauts and altered gravity inputs affecting the otolith organs could attenuate the VSR and alter blood pressure regulation. Although hypovolemia is considered to contribute to the development of postspaceflight orthostatic intolerance, its direct effect on all reflexes that contribute to blood pressure regulation during a change in posture is unknown. This study indicates that a fluid shift within the vestibular apparatus affects the VSR and might contribute to the development of overall orthostatic intolerance associated with spaceflight and aging.

ACKNOWLEDGMENTS

Authors would like to thank Jonathan Cook for technical assistance and the staff of the General Clinical Research Center for nursing support. This project was funded by grants from the National Institutes of Health (PO1HL077670, DC006459, and RR10732), National Space Biomedical Research Institute (CA00404), and the American Heart Association.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES