Intranasal scopolamine affects the semicircular canals centrally and peripherally

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Intranasal scopolamine affects the semicircular canals centrally and peripherally. J Appl Physiol 119: 213–218, 2015. First published May 7, 2015; doi:10.1152/japplphysiol.00149.2015.—Space motion sickness (SMS), a condition caused by an intravestibular conflict, remains an important obstacle that astronauts encounter during the first days in space. Promethazine is currently the standard treatment of SMS, but scopolamine is used by some astronauts to prevent SMS. However, the oral and transdermal routes of administration of scopolamine are known to have substantial drawbacks. Intranasal administration of scopolamine ensures a fast absorption and rapid onset of therapeutic effect, which might prove to be suitable for use during spaceflights. The aim of this study was to evaluate the effects of intranasally administered scopolamine (0.4 mg) on the semicircular canals (SCCs) and the otoliths. This double-blind, placebo-controlled study was performed on 19 healthy male subjects. The function of the horizontal SCC and the vestibulo-ocular reflex, as well as the saccular function and utricular function, were evaluated. Scopolamine turned out to affect mainly the SCCs centrally and peripherally but also the utricles to a lesser extent. Centrally, the most probable site of action is the medial vestibular nucleus, where the highest density of muscarinic receptors has been demonstrated and afferent fibers from the SCCs and utricles synapse. Furthermore, our results suggest the presence of muscarinic receptors in the peripheral vestibular system on which scopolamine has a suppressive effect. Given the depressant actions on the SCCs, it is suggested that the pharmacodynamic effect of scopolamine may be attributed to the obliteration of intravestibular conflict that arises during (S)MS.

utricles; saccules; acetylcholine antagonist; semicircular canals; motion sickness; space motion sickness
Over the past decades, the development of an intranasal formula of scopolamine has gained more interest since this delivery method of the drug might exhibit preventive and/or mitigating effects against SMS but with a more favorable effectiveness: the side effects ratio, its noninvasive character, ensures a rapid delivery of the drug to the blood plasma without a hepatic metabolism and results in fast onset of therapeutic actions (33). During the study described in this paper, we investigated the effects of intranasal scopolamine (0.4 mg) on the semicircular canals and the otoliths. Based on the intravestibular conflict that is responsible for triggering SMS symptoms, a pharmacologically induced suppression of the SCCs might have therapeutic actions against SMS by a neutralization of the otolith-canal conflict.

MATERIALS AND METHODS

Subjects

A total of 19 healthy male volunteers (mean age 24.4 years; range 20.15–29.32 years) were recruited for participation to the study after a medical screening that determined their eligibility. None of the participants had a history of auditory, vestibular, or neurologic deficits. The study was commissioned by the European Space Agency and approved by the institutional review board of the Antwerp University Hospital (Reference No. B3002072398). The subjects gave their written informed consent before the start of the study. All clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki.

Nasal Spray Device and Drug Administration

The nasal spray devices were manufactured under good manufacturing practices conditions by the School of Pharmacy, Department of Pharmaceutical Sciences, located at the University of Maryland, Baltimore, MD. Both placebo and scopolamine formulations were filled into Aptar bidose nasal spray devices. Each scopolamine nasal spray device delivered two puffs, with a concentration of 0.1 mg/0.1 ml per puff. To achieve a total delivery of 0.4 mg of scopolamine, two nasal spray devices (and thus a total of four puffs), one in each nostril, were administered to the subjects. For administration of placebo, the same amount of nasal sprays and puffs were applied.

A bioavailability study performed by Putcha and colleagues (23) has shown that intranasal administration of 0.4 mg of scopolamine results in peak plasma concentrations ($C_{max}$) after 0.37 ± 0.05 h and a mean residence time (average time a molecule spends in the body) of 1.57 ± 0.12 h.

Study Design

The study had a double-blind, placebo-controlled, and repeated measures protocol design. The vestibular tests were scheduled over two consecutive half days comprising a morning and an afternoon session and were always performed on the same hour of the day to exclude interfering circadian effects. Every participant took both placebo and scopolamine in random order and a washout period of at least 4 wk was foreseen. The investigator administered the product at the beginning of each session, i.e., 30 min before the start of the first vestibular assessment test to assure full absorption of the product. Alcohol consumption was not allowed one day before the first testing day and on the testing days and compliance was randomly controlled by means of a disposable alcohol test. Furthermore, all subjects were nonsmokers, and they were instructed not to consume beverages containing caffeine or quinine on the testing days. The intake of any medication other than scopolamine had to be reported to the investigator. All tests were performed under supervision of a medical doctor.

Measurements

The effects of scopolamine on the SCC, the utricles, and saccules were investigated by performing, unilateral centrifugation (UC), ocular vestibular evoked myogenic potentials (oVEMP), and cervical vestibular evoked myogenic potentials (cVEMP) tests, respectively.

Electronystagmography. Standard electronystagmographic (ENG) recording techniques are used to determine the function of the horizontal SCCs and the integrity of the vestibulo-ocular reflex (VOR). The detailed methodology and normative values are published by Van Der Stappen and colleagues (35). The ENG is recorded and analyzed by means of an 8-channel PC-based system (Nystagliner, Toennies). Testing protocol consists of several subparts. First, the presence of pathological, i.e., spontaneous or gaze-evoked, nystagmus is investigated. This is followed by an evaluation of oculomotor integrity assessed by measuring saccades, optokinetics, and smooth pursuit. Subsequently, the rotational VOR is evaluated by way of rotary chair testing performed in complete darkness. During the test, the subject is asked to close the eyes and perform a mental task, i.e., counting backwards in steps of three out loud. The applied sinusoidal rotations have a maximum velocity of 60°/s and a frequency of 0.05 Hz. The following parameters are calculated after completion of the rotary chair testing: gain (ratio of eye velocity to head velocity), phase (time lag between peak eye velocity and peak head velocity, calculated by subtracting the peak eye velocity with peak head velocity), and asymmetry. The responsiveness of the horizontal SCCs is investigated by means of caloric testing consisting of subsequent irrigations of both ears with 180 ml of warm water (44°C) and 180 ml of cold water (30°C) during 30 s. During and after the irrigations, the subject is again asked to close the eyes and to count backwards. Labyrinth and nystagmus asymmetry are calculated with the Jongkees’ formula (17), based on the maximum slow component velocities of the eye responses. Additionally, the total caloric response of the horizontal SCCs is determined by adding the four irrigations and is considered as a measure of the function of the peripheral SCC (21). All values are referenced to normative data obtained in the same setting and using the same protocol (35).

During preparation, the subject is seated in the vertical axis rotating chair (Neurokinetics) and immobilized by means of several straps and belts. Movements of the head during the rotation are avoided by means of head restraints. Subsequently, the subject is put in complete darkness except for a chair-fixed faint red light located 1 m in front of the subject, which avoids lateral deflections of the eyes and decreases the nystagmi provoked by the acceleration. After the preparation, the chair is accelerated at 3°/s² until a velocity of 400°/s is reached. On-center rotation is maintained for a fixed period of 90 s to ensure that the response of the SCCs to the acceleration has ceased. The chair is then translated alternately 4 cm to the left and right according to a sinusoidal pattern with a frequency of 0.013 Hz. During a translation to the left, the right utricular organ becomes aligned with the axis of rotation, and the left utricle is exposed to a combination of a centrifugal acceleration of 0.4 g (with g the gravitational acceleration which equals 9.81 m/s²) and a gravitational acceleration. The sum of both is called the gravito-inertial acceleration (GIA) and causes an apparent tilt of 21.7°. This GIA tilt triggers the left utricular system to induce an ocular counterrolling (OCR) which is recorded throughout the experiment by means of 3D video oculography goggles which comprises two infrared cameras and semireflective glasses reflecting the infrared waves (19).

The SCCs also contribute to the ocular torsion that occurs during the acceleration phase, and this is also taken into account in the described model. Canal function is reflected by the adaptation time constant (i.e., time constant characteristic for the SCC contribution to OCR), cupular time constant (time constant characteristic for the
mechanical recoil of the cupula), velocity storage time constant, and the maximal canal response to OCR. Utricular integrity is characterized by the bias (i.e., utricular mediated offset of OCR corrected for the offset induced by the SCC), gain (ratio of the response (OCR) to the stimulus (GIA tili)), and the absolute asymmetry (dominance of left over right utricule). All values are referenced to normative data, obtained in the same setting, and using the same protocol (4).

Ocular vestibular evoked myogenic potentials. Recently, the oVEMP test has been introduced as a new tool for utricular function evaluation and has gained much interest ever since (5, 27).

Application of bone-conducted vibrations on the forehead at the Fz position, i.e., the junction of the midline of the face and the hairline, is believed to trigger the utricular organs bilaterally. This stimulation generates a crossed excitatory response that travels via the utricular-ocular reflex to the inferior oblique muscle, where the response is being measured. The response has a biphasic shape with a negative peak at 10 ms (n10) followed by a positive peak at 15 ms (p15) (6, 13).

At the start of the test, subjects are asked to lie supine on a bed with their head positioned horizontally on a pillow. The following electrodes (Ambu Blue sensor N, Ambu A/S, Ballerup, Denmark) are applied by the investigator: two active electrodes just above the infraorbital ridge of both eyes, two reference electrodes next to the nostrils, and the ground electrode on the sternum. Before the actual measurement starts, a calibration is performed and participants are instructed to look upward during 10 s to a red cross on the ceiling. This generates an upward eye movement of ~30°, bringing the inferior oblique muscle to the surface beneath the eye. The electromyographic values of the inferior oblique muscle are determined twice for each eye. The ratio of these values will be used after the test to correct the recorded peak-to-peak amplitudes. The actual oVEMP stimulation is performed by means of a hand held mini shaker (Bruel and Kjaer, Type 4810. Naerum, Denmark) that produces tone bursts of 500 Hz lasting 6 ms. Subjects are again asked to look at the red cross on the ceiling during the application of the vibrations. First, the repetition rate of the vibration is set at 3 stimuli per second (3 Hz), followed by a vibration consisting of 11 stimuli per second (11 Hz), which is repeated, and finally the measurement is ended with a second stimulation at 3 Hz.

The following parameters are determined for each eye at all repetition rates: n10 and p15 latencies, latency differences between left and right, and peak-to-peak amplitudes. The peak-to-peak amplitudes measured at 11 Hz are averaged and used to calculate the interocular ratio and the corrected interocular ratio by means of the following equations:

\[
IAR = \frac{ampl (L) - ampl (R)}{ampl (L) + ampl (R)} \times 100
\]

\[
cIOR = \frac{ampl (L) - ampl (R)}{ampl (L) + ampl (R)} - \frac{EMG (L) - EMG (R)}{EMG (L) + EMG (R)} \times 100
\]

Cervical vestibular evoked myogenic potentials. Saccular function is evaluated by means of the cVEMP test. The saccular organ is triggered by air-conducted tone bursts (500 Hz) and generates, via the sacculocollic pathway, an inhibitory response that is measured at the ipsilateral sternocleidomastoid (SCM) muscle. The recorded response has a typical biphasic shape with a positive peak after 13 ms (p13) and a negative peak after 23 ms (n23). Since the amplitude (peak-to-peak) of the cVEMP response depends on sound intensity and muscle contraction, it is important that the SCM muscle is contracted in a consistent manner. Therefore, the subject is asked to push the head against his hand to contract the SCM muscle. Feedback about the strength of the contraction is provided on a monitor displayed on a computer screen located next to the subject (34). Active electrodes (Ambu Blue sensor N, Ambu A/S) are placed in the center of left and right SCM muscles; the reference electrode is applied on the sternum and the ground electrode on the forehead.

Tone bursts are offered unilaterally to both ears by means of an insert earphone (E-A-Rtone Gold, E-A-R). Sound intensity is first set at 95 dB SPL and lowered with 5 dB SPL until no cVEMP waveform can be recorded anymore. If no response is measured at 95 dB SPL, sound intensity is increased to maximal 100 dB SPL. The threshold is considered as the lowest intensity that still elicits a response. For each ear, a total of two series are performed. The parameters characterizing saccular function are based on the average of the two responses measured at 95 dB: the peak-to-peak amplitude, peak-to-peak latencies, and latency differences between the right and left saccule. For the peak-to-peak amplitude at 95 dB SPL, the asymmetry between left and right saccular system is determined by means of the interaural ratio (IAR) and the corrected IAR (cIAR), where the correction takes the variance in muscle contraction into account by means of (i.e., mean rectified voltage). Both are calculated by means of following equations:

\[
IAR = \frac{ampl (L) - ampl (R)}{ampl (L) + ampl (R)} \times 100
\]

\[
cIOR = \frac{ampl (L) - ampl (R)}{ampl (L) + ampl (R)} - \frac{MVR (L) - MVR (R)}{MVR (L) + MVR (R)} \times 100
\]

Statistical Analysis

Outcome variables of the different vestibular assessment tests were determined for both placebo and scopolamine. Effect of scopolamine vs. placebo was tested using a paired samples t-test. For all comparisons, a significance of \( P < 0.05 \) was chosen. All statistical analyses were performed with SPSS V.20.

RESULTS

ENG

The VOR characteristics phase and asymmetry, obtained during rotary chair testing, were omitted from the database when VOR gain values were lower than 0.20, since these values are no longer reliable. This resulted in the removal of VOR characteristics of two subjects for placebo and five subjects for scopolamine.

Statistical analysis of the parameters recorded during ENG revealed a significant decrease of the VOR gain (paired t-test, \( P = 0.023 \)) (Fig. 1 and Table 1) postscopolamine. Furthermore, a significant decrease of the total caloric response (TCR) (hot and cold values combined) was observed after scopolamine.
intake compared with placebo (paired t-test, \(P = 0.003\)) (Fig. 2 and Table 1). No other significant effects were observed on the remaining ENG outcomes.

**UC, cVEMP, and oVEMP**

Outcomes of the UC test could not be calculated for one subject during the intake of scopolamine because the raw data were of bad quality (e.g., this could be caused by a tear in the eye, blurring the structure of the iris).

Statistical analysis revealed a significant increase after scopolamine intake of the peak-to-peak amplitude of cVEMP response measured at the right side (paired t-test, \(P = 0.033\)) (Fig. 3 and Table 2). Also, the corrected peak-to-peak amplitude was significantly increased postscopolamine at the right side (paired t-test, \(P = 0.036\)). The strength of the SCM muscle contraction at the right side did not differ significantly between scopolamine and placebo (paired t-test, \(P = 0.630\)). There were further no significant results on the parameters of the cVEMP.

No significant effects of scopolamine on the outcomes of the UC and oVEMP were observed after comparison with placebo (paired t-test). However, a decreasing trend of the gain of the OCR was observed; however, the effect was not significant.

This newly acquired knowledge regarding the effects on the SCCs adds to the current insights regarding the possible site of actions of scopolamine. Scopolamine is an antimuscarinic agent and is nonselective for the five types of muscarinic receptors. Muscarine binding sites have been identified in all the vestibular nuclei with the highest density in the medial vestibular nucleus (MVN), and stimulation of these receptors increases neuronal activity (28). Furthermore, second-order neurons transferring information about movements to the cerebellum, including the flocculus and the nodulus, have been shown to be acetylcholine (ACh) sensitive, and researchers have postulated that this pathway might play an important role in MS-relieving effects of anticholinergics (2, 3). Finally, it has been hypothesized that ACh is also implicated in afferent fibers and efferent brainstem projections to the hair cells. However, the exact modulatory role, i.e., inhibition or excitation, of efferent ACh is not entirely clear since both have been reported (8–10). Anticholinergic agents are believed to act on the central vestibular system (32).

Based on the findings of the present study, i.e., a significant decrease of the VOR gain and a significant decrease of the total caloric responsiveness, a combined central and peripheral action of the drug is hypothesized. A study performed by Pyykkö...
Table 2. Descriptive statistics of significant cVEMP and UC outcomes

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SE</th>
<th>SD</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude ri, μV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>237</td>
<td>23</td>
<td>98</td>
<td>188</td>
<td>286</td>
<td>0.033</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>285</td>
<td>28</td>
<td>120</td>
<td>225</td>
<td>344</td>
<td></td>
</tr>
<tr>
<td>Gain UC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>0.25</td>
<td>0.02</td>
<td>0.07</td>
<td>0.21</td>
<td>0.28</td>
<td>0.056</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>0.20</td>
<td>0.01</td>
<td>0.06</td>
<td>0.17</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

Effect of scopolamine on the response amplitude of the right saccule and on the gain of the ocular counterrol (OCR) in the 19 subject. The mean value (±SE) of the amplitude is represented for placebo and scopolamine. Scopolamine signficantly increased the amplitude of the response amplitude of the right saccule.

Earlier studies in mice, rats, and turtles (12, 18, 22). Muscarinic receptors on the vestibular afferents contribute to the excitability of the vestibular peripheral sensors. Adding scopolamine will lead to a suppression of these muscarinic receptors, therefore reducing the vestibular afferent input and leading to an alleviation of the sensory mismatch. However, efferent inputs could still contribute to the vestibular mismatch by trying to recalibrate the sensitivity of the end organs in the face of a constant, novel vestibular stimulus. We cannot, however, minimize or discount central muscarinic mechanisms, in particular in the vestibular nuclei, since they are still potential targets.

Outcomes from the cVEMP test showed a significant increase of the (corrected) peak-to-peak amplitude of the response recorded after stimulation of the right saccule. This effect did, however, not manifest on the response amplitude of the left saccule or on the IAR. Since the contraction of the SCM muscle has an influence on the peak-to-peak amplitude, an increased strength of the contraction of the right muscle during intake of scopolamine might have caused the amplitude to be higher. It seems counterintuitive that only one and not both saccules were affected and that the actions of scopolamine are enhancing rather than suppressive. Therefore, the increase of the right c-VEMP seems coincidental and the effect of scopolamine on the amplitude of the right saccule was considered as clinically not significant.

Further results from the cVEMP test suggest that scopolamine did not affect saccular function. It is argued that the agents might not affect the nucleus receiving saccular information. Indeed, target receptors of the drugs are predominantly located in the MVN, whereas the majority of saccular fibers have been shown to terminate in the inferior vestibular nucleus. However, it should be emphasized that the lack of effects might also be a consequence of the technique and methodology used. It seems probable that the cVEMP as well as the oVEMP tests are not sensitive enough to allow them to identify pharmacological effects in a healthy population.

Indeed, it should be emphasized that no definite conclusions can be drawn regarding the SMS-relieving effects of scopolamine, as this can only be achieved by performing efficacy studies during ground-based simulations of SMS or during spaceflight.

In conclusion, the findings in this study postulate that scopolamine exerts its effects on the function of the SCCs, possibly by a central inhibitory action on the MVN, and it may also affect the efferent or afferent fibers located in the peripheral vestibular system. The effects of the drug on the utricular reflex function were rather small and nonsignificant. Since SMS is believed to be caused by an intravestibular conflict, suppression of the canals might abolish that conflict and thus
relieve SMS symptoms. Furthermore, given the fact that scopolamine is considered to be the most efficient drug against MS and that the nasal delivery system has been proven to be a fast and efficient route of administration, it makes the drug an interesting candidate to be further evaluated for its potentials against SMS.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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