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Running head

Effects of selective muscarinic receptor antagonist (Darifenacin) on motion sickness

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Abstract

Aims: The aim of this study was to compare the effects of the selective M3 muscarinic acetylcholine receptor antagonist Darifenacin, oral Hyoscine hydrobromide and Placebo on motion sickness induced by cross-coupled stimulation.

Methods: The effects of Darifenacin 10 mg or 20 mg, Hyoscine hydrobromide 0.6 mg and Placebo were assessed in a randomised, double-blind, 4-way cross over trial of 16 healthy subjects. Motion sickness, skin conductance (a measure of sweating) and psychomotor cognitive function tests were investigated.

Results: Hyoscine hydrobromide produced significantly increased tolerance to motion versus Placebo ($P<0.05$ to $P<0.01$). The motion protection effect of Darifenacin (10 or 20 mg) was approximately one third of that of Hyoscine hydrobromide, but was not significant versus Placebo**.** Darifenacin and Hyoscine hydrobromide both significantly reduced skin conductance versus Placebo. Darifenacin produced either no effect or an enhanced effect on cognitive function in contrast to Hyoscine hydrobromide where there was significant impairment of psychomotor performance.

Conclusion: The results suggest that selective antagonism of the M3 receptor may not be important in the prevention of motion sickness. However selective M3 antagonism does not impair cognitive function. These observations may be important given that long term treatment with non-selective anti-muscarinic agents such as Oxybutynin may lead to an increased incidence of dementia.

What is already known about this subject

- Recent observational studies have demonstrated that long term usage of a non-selective anti-muscarinic antagonist is associated with an increased incidence of dementia.
- Short term usage of Hyoscine hydrobromide for the treatment of motion sickness is known to be associated with drowsiness and other CNS side effects compatible with a direct effect on M1 & M2 receptor sub types.

What this study adds

- The study has shown that there are important differences in clinical effects produced by selective and non-selective antagonists on both motion sickness and cognitive function respectively.
- The study demonstrates that selective M3 antagonists may enhance certain aspects of cognitive function and have a neutral effect on other domains.
- The loss of an effect on motion sickness seen with the M3 selective anti-muscarinic agent Darifenacin suggests that the M3 receptor subtype is not relevant in the prevention of motion sickness.

Introduction

Hyoscine hydrobromide, which is effective in the prophylaxis of motion sickness [1] shows similar binding affinities to all of the five known muscarinic acetylcholine receptor sub-types [2]. However, Hyoscine hydrobromide can produce a number of unwanted side effects including blurred vision, drowsiness and impaired psychomotor performance [3-5]. The development of selective muscarinic receptor antagonists with central actions leads to the possibility of enabling motion sickness protection with reduced side effects, provided that there is a functional separation between the roles of central muscarinic receptor subtypes. Darifenacin is a selective muscarinic M3 receptor antagonist with good selectivity over atrial M2 and neuronal M1 receptors. Animal studies confirm central nervous system (CNS) penetration by Darifenacin following oral administration [6].

Darifenacin is approved for the treatment of urge urinary incontinence (UUI). Short-term studies have shown that commonly used non-selective anti-muscarinic drugs such as Oxybutynin, have a detrimental effect on cognitive function [7].

We have compared the effects on motion sickness elicited by cross-coupled stimulation of Darifenacin and of Hyoscine hydrobromide. In addition, the effects on sweating measured by skin conductance (SC) and on a variety of aspects of psychomotor and cognitive function were evaluated.

Methods

Study Design

This was a randomised, Placebo controlled, double-blind, 4-way cross-over study. Over 4 study sessions the subjects were dosed with single oral doses of Darifenacin 10 mg, Darifenacin 20 mg, Hyoscine hydrobromide 0.6 mg, or Placebo. The order of administration of the 4 treatments was counterbalanced between subjects using a Latin Square design.

Each study session had 2 stages. On the first the Motion Challenge described below was conducted 90 minutes after treatment administration. SC was also measured. In order to avoid any problems of timings or effects and interactions of cognitive performance with motion sickness, the various CNS assessments including self-ratings and the performance of cognitive tests were conducted on a separate occasion at least 48 hrs following the motion sickness study. The same dosing condition was administered.

On the treatment days the drugs were administered with 200ml of water. Subjects were not required to fast overnight prior to study drug administration, however no heavy meals were allowed the night before and no food consumption was allowed one hour before drug administration. Subjects were required to not consume any alcohol for 24 hours, and to not smoke for one hour before treatment.

The Darifenacin used in the study was an immediate release formulation administered orally in a capsule. For the purposes of this study two doses were prepared. Capsules contained either 10 mg or 20 mg of Darifenacin. Matching

Placebo and Hyoscine hydrobromide 0.6 mg capsules were also provided by Pfizer (Tadworth, United Kingdom).

The wash-out period between treatments was 48 hours and deemed acceptable, given that the terminal half-life ($t_{1/2}$) of Darifenacin and Hyoscine hydrobromide was 3.5-4.5 hours and 1-4.5 hours, respectively.

The doses of these drugs were determined on the basis of previously published data. Hyoscine hydrobromide 0.6 mg is the standard dose for these types of clinical studies and the pharmacokinetic profile of this dose has been well studied [8].

The immediate-release (IR) formulation of Darifenacin (10 mg and 20 mg) showed similar effects on the bladder as the IR Oxybutynin [9], albeit produced less dry mouth. The pharmacokinetic properties of the IR and CR Darifenacin were similar with respect to t_{max}, albeit IR Darifenacin has an increased peak to trough ratio compared to CR [9-12].

Subjects

Subjects were sixteen healthy male volunteers (mean 26.47 years; S.D. 7.94, range 17-43 years) with intact vestibular function and not currently on other medications. Prior to any study related procedures taking place, the subjects were fully informed of the procedures involved in this study and the risks of taking these drugs and their informed consent was obtained. Subjects were free to withdraw from the study any time. This study was approved by the Kent and Canterbury Hospital Ethics committee. During the consent and screening

process the subjects' eligibility, medical history, medication use and fitness was thoroughly assessed by the investigators. Subjects were given sufficient time to read the 'Information for Volunteers' document and make an informed decision. All information provided by subjects was treated with confidentiality and integrity. All trial related activities were carried out in accordance with ICH GCP Guidelines and Declaration of Helsinki 1989.

Procedures

Prior to the start of the study, a familiarisation session was conducted with the motion challenge and SC assessment. On another study session, the volunteers undertook the various CNS assessments on four repeated occasions to remove any training effects [13].

On the study days involving the motion challenge, the study medication was administered 90 minutes prior to initiation of the challenge. On the cognitive function assessment days, the tasks were administered on arrival, prior to study treatment administration, and then again at 1.5, 3 and 4.5 hours after dosing.

Motion Challenge

Motion sickness was elicited by cross-coupled stimulation on a turntable, with subjects blindfolded. The rotational velocity was incremented by 2 deg/sec every 30 sec, and a sequence of eight head movements of 45° ware completed every 30 sec [14]. Motion was stopped at moderate nausea, or 40 minutes,

whichever occurred sooner. Subjects rated their degree of motion sickness on the following scale: 1 = no symptoms; 2 = Any symptoms however slight; 3 = Mild symptoms, e.g. stomach awareness but no nausea; 5 = Mild to moderate nausea; 6 = Moderate nausea but can continue; 7 = Moderate nausea, want to stop motion challenge [15]. At the end of motion, a sickness symptom checklist was administered for: Dizziness, Bodily warmth, Headache, Sweating, Stomach awareness, Increased salivation, Funny taste in mouth, Dry mouth, and Nausea (symptom scoring Nil=0, Mild=1, Moderate=2, Severe=3).

Skin Conductance

The skin conductance (SC) method employed was similar to that developed by Golding [14]. SC was recorded from 1st and 2nd finger palmar sites of the non-dominant hand, and from the left and right sides of the forehead close to the hairline approximately 2 to 4 cm above the eyebrows (supra-orbital ridge). Ag/AgCl electrodes were attached with double-sided self-adhesive stickers. Total effective electrical skin contact area was 0.32 cm^2 for the 2 finger electrodes and 0.32 cm^2 for the 2 forehead electrodes. The electrolyte used was 0.05 M NaCl, in the range found in human sweat [16]. The electrolyte jelly was made up with carboxymethylcellulose as the gel agent: NaCl 0.3g plus low substitution carboxymethylcellulose 5g (BDH Ltd) plus water to 100g total [17]. SC was measured using two constant current (10 µA) mains-isolated devices built in the laboratory. Output was recorded in terms of DC-coupled (tonic) SC level (SCL) and as the amplified AC-coupled high pass signal (phasic) SC

responses (SCRs) (corner frequency 0.14 Hz). Following 12 bit A/D conversion at 5 Hz with anti-aliasing low pass filter above 2 Hz, the SCL and SCR signals were displayed online and stored on hard disk with automatic diskette back-up. SCR expressed as μmho RMS was subsequently analysed in the frequency band 0.005 - 0.48 Hz.

Tests of CNS Function

The volunteers were trained on the cognitive function assessments prior to the main study. Each volunteer completed all the procedures four times during this training. Subsequently on each study day in which cognitive function was assessed, the volunteers performed the tests one hour prior to dosing and at 1.5, 3.5 and 4.5 hours afterwards. The cognitive function tests took approximately 25 minutes to complete. They were preceded by a mood and alertness questionnaire. This questionnaire comprised sixteen 100 mm visual analogue scales which were combined as recommended by Bond and Lader [18] to derive 3 factors: subjective alertness, subjective calmness and subjective contentment. The questionnaire has proven sensitive to a wide range of compounds [19-21].

A selection of tasks from the Cognitive Drug Research (CDR) Computerised Cognitive Assessment System [22-25] was performed. The tests were administered in the following order:

1. Word presentation
2. Immediate word recall
3. Simple reaction time

4. Digit vigilance task
5. Choice reaction time
6. Tracking
7. Numeric working memory task
8. DSST
9. Delayed word recall
10. Word recognition task
11. Critical flicker fusion frequency (CFF)
12. Body Sway: This was assessed using the Wright Ataxiameter

On each testing session, parallel forms were administered, where appropriate (i.e. different lists of words different sequences of digits and different random sequences in tasks where the order of stimuli is randomised). All tasks were computer controlled, the information being presented on high resolution monitors and then responses recorded via response modules containing two buttons, one marked "NO" and the other "YES" [22-25].

Statistical analysis

Following an initial scrutiny of the motion sickness data, checks were performed for appropriate symptom patterns, and for significance of order effects. Internal consistency checks were performed on times to motion endpoint between practice and Placebo sessions, and between individual symptom items. Analyses were carried out to examine the effects of systematic exclusions of

subject/treatment combinations where no nausea was reported at endpoint, or where the symptom pattern was highly suspect e.g. nausea but no stomach awareness, i.e. to exclude those data where motion was terminated at a nominal sickness rating of 7 but where the meaning of this rating was open to doubt.

Two minutes blocks of SC data were extracted from the beginning of the motion challenge, i.e. well prior to the onset of initial symptoms of motion sickness, and two minutes blocks of SC data were taken at the end of the motion challenge, i.e. during the period of time of maximum motion sickness. The differences between the first and last 2 minutes block of each finger and head SC dataset were addressed as a time factor in this analysis. A preliminary analysis of variance (ANOVA) was performed on the log-transformed dataset to examine whether there was any significant run order effect. No such run order effect was found, and therefore the analysis proceeded to address the treatment and time effects. ANOVA was applied to partition the overall variation in the log-transformed dataset, according to time, treatment, and the 2 way interaction between them. Newman-Keuls Tests were applied to assess the differences between the individual treatments for each SC channel. In addition to ANOVAs, non-parametric tests (Wilcoxon) were used as appropriate.

For the cognitive function data the statistical analysis was performed as follows; all data was adjusted for the pre-dosing scores on a study day to derive 'difference from baseline scores'.

Results

Motion Challenge

Visual scrutiny of times to sickness ratings revealed an appropriate pattern of symptoms, therefore an initial analysis of the data was made using ANOVAs and non-parametric (Wilcoxon) test which would be less sensitive to extreme responses than parametric analysis. Times to sickness rating 7 were significantly longer for Hyoscine hydrobromide compared with Placebo (P=0.027 2-tailed Wilcoxon Test), but Darifenacin at either dose was not significantly different from Placebo (Figure 1, Table 1).

Data were identified for exclusion from the subsequent analysis by ANOVA by scrutinising for treatment/subject combinations which were unusual in terms of the expected associations between nausea and stomach awareness for symptom scores at motion endpoint, or typically where low nausea was recorded at motion endpoint (Supplementary Table 2). In the latter event it was possible that a subject may have stopped for other reasons such as dizziness.

A series of ANOVAs used these systematic exclusions of subject/treatment combinations. The majority of Hyoscine hydrobromide versus Placebo comparisons were significant (21/32 comparisons at $P<0.05$; 15/32 comparisons at $P<0.001$), whereas none were significant for Darifenacin (10 or 20 mg) versus Placebo.

On average Hyoscine hydrobromide 0.6 mg produced an increased motion tolerance over Placebo of around 3 min, whereas Darifenacin 10 or

20 mg produced an increased motion tolerance over Placebo averaging around 1 min (Figure 1).

Symptom scores at motion endpoint

ANOVA of symptom scores at motion endpoint revealed significant treatment effects on dry mouth, salivation and bodily warmth. Dry mouth was significantly higher for Hyoscine hydrobromide ($P<0.05$) and for Darifenacin (10 mg $P<0.05$, 20 mg $P<0.001$), versus Placebo. Salivation and bodily warmth were both significantly lower ($P<0.05$) with Darifenacin 20 mg versus Placebo. Symptom scores at motion endpoint are presented in Figure 2.

Skin Conductance

An example SC recording is shown in Figure 3. Figure 4 presents example palmar phasic SC records for the four treatment conditions, prior to motion sickness. Figure 5 presents the mean SC activity for the initial 2 min sample pre-motion sickness and the final 2 min sample during motion sickness

For finger SC, the mean log signal power for the last 2 minutes block was larger, to a small but significant extent, than that for the first 2 minutes ($F(1,15)=4.922$, $p<0.05$). There was also a significant treatment effect ($F(3,15)=8.099$, $p<0.01$). All three drug treatments gave significantly lower mean log signal power than did Placebo, but the three drug treatments were not significantly different one from another. The overall difference between 10 mg

Darifenacin and Placebo, and between 0.6 mg Hyoscine hydrobromide and Placebo were both significant at $p<0.05$. The overall difference between Placebo and 20 mg Darifenacin was significant at $p<0.01$. Specific comparisons of each drug treatment versus Placebo were performed for equivalent time periods, in order to further isolate effects. These are shown in Figure 5.

For forehead SC, the mean log signal power for the last 2 minutes block was significantly greater than that for the first 2 minutes ($F(1,33)=17.999$, $p<0.001$). The treatment effect was marginal ($F(3,15)=2.951$, $p=0.06$). The overall difference between Placebo and 20 mg Darifenacin showed a significantly lower mean log power for Darifenacin, at $p<0.05$. Specific comparisons of each drug treatment versus Placebo were performed for equivalent time periods, in order to further isolate effects. These are shown in Figure 5.

Cognitive Function Testing

Oral Hyoscine hydrobromide significantly reduced the accuracy of choice reaction time performance and increased body sway. A number of trends for impairment were detected, particularly at 4.5h post dosing where vigilance accuracy was decreased, tracking error increased and performance on the digit symbol substitution tests and critical flicker fusion tests impaired. When Hyoscine hydrobromide was compared directly to Darifenacin, performance with Hyoscine hydrobromide was consistently found to be inferior to that with one or both Darifenacin doses. At 1.5h post dosing, significant impairments with Hyoscine hydrobromide were seen on simple reaction time, body sway, vigilance

accuracy, DSST performance, CCF tracking, working memory sensitivity and subjective alertness. The only clearly negative effect of Darifenacin was for both doses to significantly increase body sway 4.5h post-dosing.

Discussion

The aim of this experiment was to investigate the effects of Darifenacin and Hyoscine hydrobromide on motion sickness, sweating and psychomotor performance. At the dose used, Hyoscine hydrobromide produced a significantly increased motion tolerance over Placebo of around 3 min, whereas Darifenacin 10 or 20 mg non-significantly increased motion tolerance over Placebo by around 1 min. To place these motion sickness protection values in context, a previous trial [8] using comparable methods but different subjects [26] demonstrated that Hyoscine hydrobromide 0.6 mg gave a significantly increased tolerance over Placebo of 2.9 min. Thus the results for Hyoscine hydrobromide in the present trial (3 min) were consistent with what might be expected from other evidence using this method.

Any putative anti-motion sickness effect for Darifenacin in the dose range here would be small by comparison to Hyoscine hydrobromide 0.6 mg. Given the negative evidence for the role of M1 or M2 receptors [27] and positive evidence (Zamifenacin) for a role for M3/m5 receptors in mediating anti-motion sickness actions [8], the negative results of this experiment for M3 point to a more important role for the m5 receptor. A role for the M4 autoreceptor in motion sickness is also possible.

Darifenacin significantly reduced ongoing SC activity at the fingers before motion sickness, as did Hyoscine hydrobromide. The Darifenacin induced reduction of SC activity, across the dose range employed, was similar in magnitude to that produced by Hyoscine hydrobromide. Although the higher dose of Darifenacin appeared to give the larger effect, a dose response effect between 10 mg and 20 mg of Darifenacin could not be demonstrated at a level of statistical significance. A broadly similar pattern of effects was observed at the finger sites during motion sickness.

Forehead sweating at baseline was minimal, which unsurprisingly was not significantly further reduced by the drugs. SC activity increased significantly during motion sickness, particularly at the forehead recording site, a finding consistent with previous work [14]. Darifenacin and Hyoscine hydrobromide significantly reduced this activity. A dose response effect between 10 mg and 20 mg of Darifenacin could not be demonstrated at a level of statistical significance, although the higher dose of Darifenacin appeared to give the larger effect by comparison with Placebo.

The reduction of forehead sweating during motion sickness observed with Darifenacin or Hyoscine hydrobromide was probably due to a direct drug action on sweating. The possibility of drug action on SC activity via CNS effects cannot be ruled out entirely since the drugs were given systemically. The muscarinic postganglionic innervation of the sweat gland is thought to be of the M3 type [28], suggesting that the action on SC activity of either drug is by virtue of their actions at such receptors.

Subjective symptoms of dry mouth were significantly higher for Hyoscine hydrobromide and for Darifenacin versus Placebo. Salivation and bodily warmth were both significantly lower with Darifenacin 20 mg versus Placebo. Such effects are consistent with the well-known profile of action of anti-muscarinics on secretory glands and are probably occurring at the M3 receptor [28]. This pattern of response would also be consistent with the significant sweating reduction noted above for Hyoscine hydrobromide and Darifenacin on the SC measures.

When Hyoscine hydrobromide was compared directly to Darifenacin, performance with Hyoscine hydrobromide was consistently found to be inferior to that with one or both of the Darifenacin doses. At 1.5h post dosing, significant impairments with Hyoscine hydrobromide were seen on simple reaction time, body sway, vigilance accuracy, DSST performance and subjective alertness. At 4.5h, similar significant effects were seen on vigilance accuracy, DSST performance, CFF tracking, working memory sensitivity and subjective alertness.

Darifenacin was largely free of impairing effects on cognition, in fact performance appeared to be improved by the drug. Both doses significantly improved simple reaction time and the low dose significantly improved working memory sensitivity, subjective alertness and subjective contentment. The high dose showed improved choice reaction time while the low dose showed a similar trend for improving vigilance reaction time. The only clearly negative effect of Darifenacin was for both doses to significantly increase body sway 4.5h post-dosing. The absence of an increase in tracking error with Darifenacin strongly suggests that this was not an effect on co-ordination and thus the basis for this

effect on postural stability is unclear. The magnitude of the effect is in the region of that produced by 2-3 units of alcohol and thus is clearly not a marked effect. These findings consistently demonstrate that subjects will be better off in terms of their ability to concentrate and conduct skilled activities when taking Darifenacin than when taking Hyoscine hydrobromide at the doses used in this study.

Overall the present study has demonstrated that when the volunteers received Hyoscine hydrobromide, their general ability to maintain attention and concentrate was inferior to that when they were given Darifenacin. This is in part due to the impairments produced by Hyoscine hydrobromide, but exaggerated in some cases by unexpected improvements seen with Darifenacin. Nonetheless, the outcome is that volunteers would clearly be in a better position to drive and conduct activities requiring high levels of concentration and co-ordination when taking Darifenacin than while taking Hyoscine hydrobromide.

Hyoscine hydrobromide produced significant decrements in psychomotor performance which were similar to previous published data [4]. Darifenacin produced little or no impairment at either dose or even an enhanced effect on certain parameters, suggesting that such impairments produced by Hyoscine hydrobromide are not mediated by M3 receptors.

Muscarinic receptors expressed in the brain modulate a multitude of signaling pathways involved in critical functions including attention, memory and learning. Quantitative analysis studies have identified the five subtypes with varying expression levels throughout the brain. M1, M2 and M4 are the dominant subtypes, while M3 and M5 are found at lower levels [29].

M1 receptors are of particular interest in the cortical areas and the hippocampus [30] as it has been shown that their inhibition leads to impaired spatial memory [29]. Molecular manipulations of the m1 gene have been shown to cause cognitive deficits [31], whereas the administration of known agonists of the M1 receptor subtype in human subjects with Alzheimer's disease and schizophrenia improved memory and learning [32, 33]. These findings indicate that M1 receptor antagonism causes adverse reactions in the CNS [34].

Delivery of drugs to the CNS depends on molecular characteristics; these include molecular size, polarity and lipophilicity (Supplementary Table 3) [7, 8, 35-38]. The physicochemical properties of Oxybutynin and Hyoscine hydrobromide grant these molecules high propensity to penetrate the blood-brain barrier freely, thus high concentrations can reach the central M1 receptors [39].

The affinity of anti-muscarinics for specific receptors is an important factor contributing to CNS effects. Oxybutynin has been shown to have similar selectivity for M1 and M3 receptors (pK_i 8.7 and 8.9, respectively) and is considered non-selective [30]. Short term studies have demonstrated impairment of cognitive function after Oxybutynin therapy [40]. A recent observational study has shown that chronic usage of Oxybutynin in patients with UUI is associated with a significant increase in the incidence of dementia [41]. The positive cognitive function data shown in this study should be confirmed in long-term studies in patients with UUI treated with Darifencin. It would seem axiomatic to avoid chronic usage of anti-muscarinic agents that result in adverse effects on cognitive function which may not be reversible and lead to dementia.

We conclude that the muscarinic M3 receptor is unlikely to be the most important receptor mediating the anti-motion sickness actions of Hyoscine hydrobromide. This result, in conjunction with other data, indicates a more important role for the m5 receptor in motion sickness. In addition, this study suggests that selective antagonism of the M3 receptor does not produce a deleterious effect on cognitive function. This observation may be important when considering selection of a suitable anti-muscarinic agent for long term treatment of chronic conditions such as UUI.

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565

Figure legends

Figure 1. – Mean times to sickness rating 7 "moderate nausea & wish to stop" (motion endpoint) for the three active treatments and Placebo, repeated on n=15 subjects completing all treatments. Motion sickness was elicited by cross-coupled stimulation on a turntable. The mean times shown are representative of the range of results produced by exclusions on the basis of statistical symptom pattern tests, or on the basis of contradictions between nominal sickness rating and recorded symptoms such as nausea and stomach awareness at motion endpoint. The majority of Hyoscine hydrobromide (HBr) 0.6 mg versus Placebo comparisons were significant ($P<0.05$ to $P<0.001$), whereas none were significant for Darifenacin (10 or 20 mg) versus Placebo.

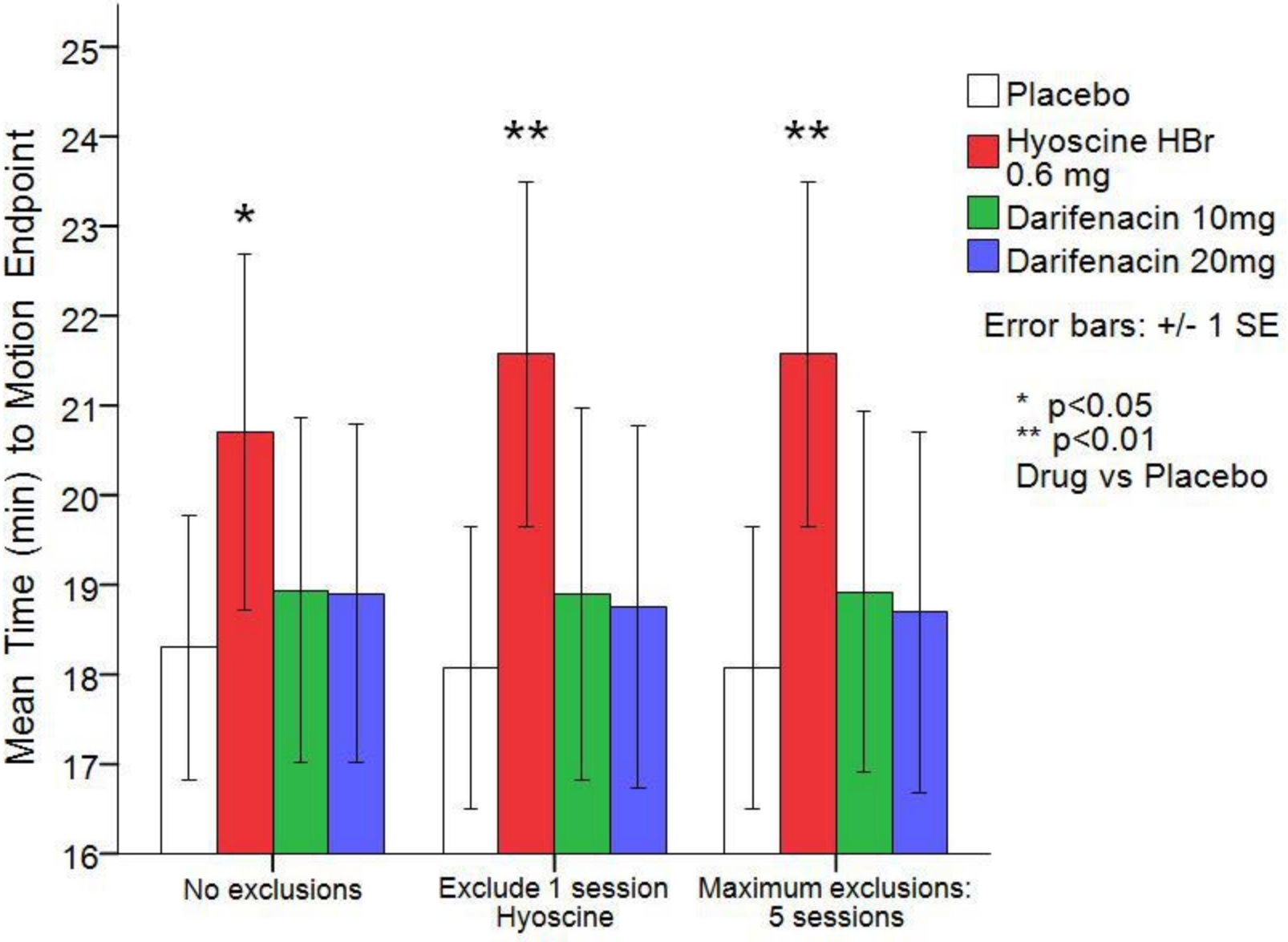
Figure 2. – Mean Symptom scores (0=nil, 1=mild, 2=moderate, 3=severe) are shown at the motion endpoint of sickness rating 7, for n=15 subjects completing all treatments. The most consistent effect was that Darifenacin and Hyoscine hydrobromide (HBr) increased subjective dry mouth.

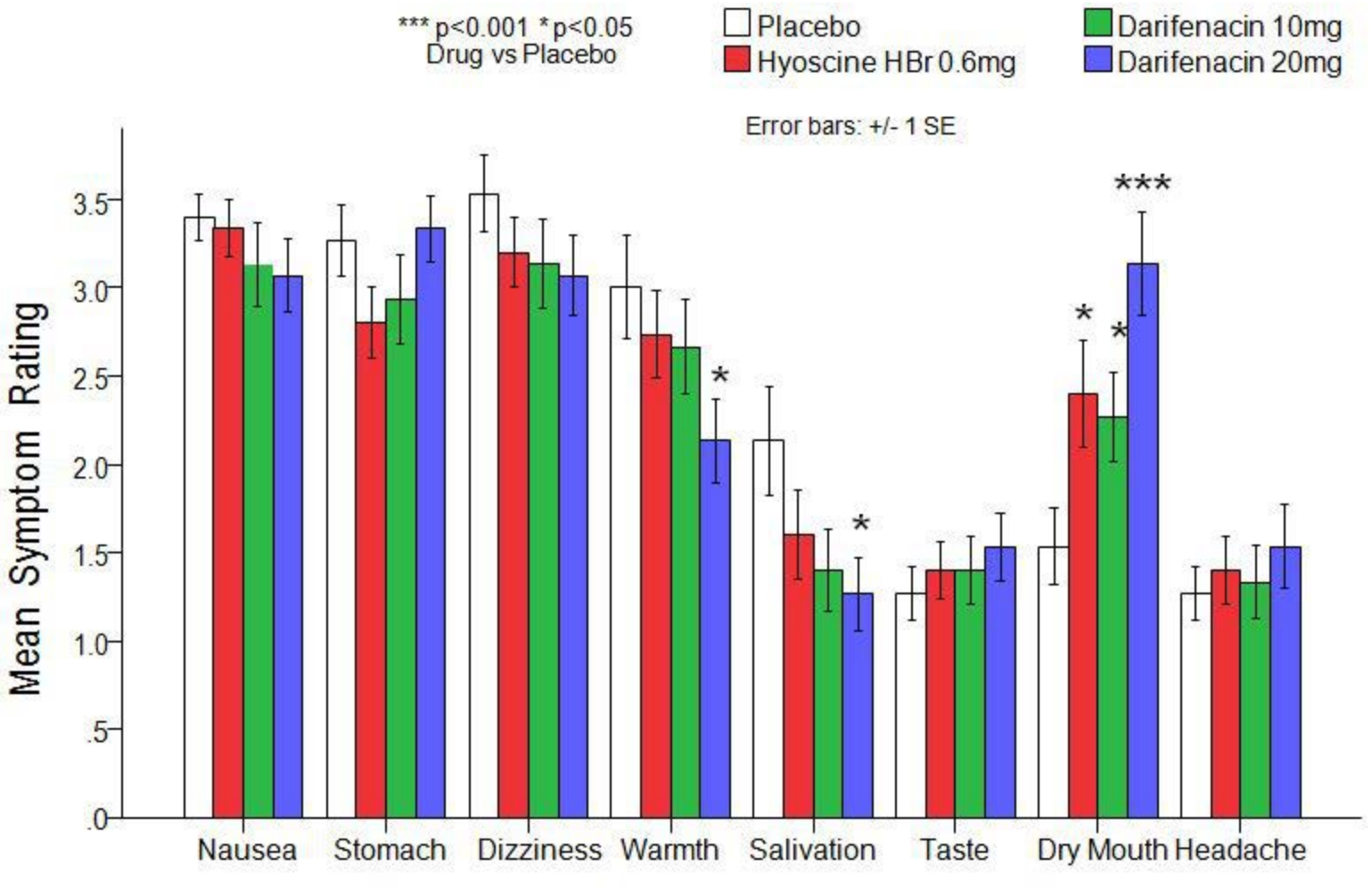
Figure 3. – Skin conductance recordings at forehead and finger palmar sites during motion sickness challenge show finger palmar site being active throughout the test, showing non-specific response, whereas the forehead site is showing rapid increase at the onset of moderate nausea, as reflected in the Increase in Sickness rating, at approximately 13 minutes.

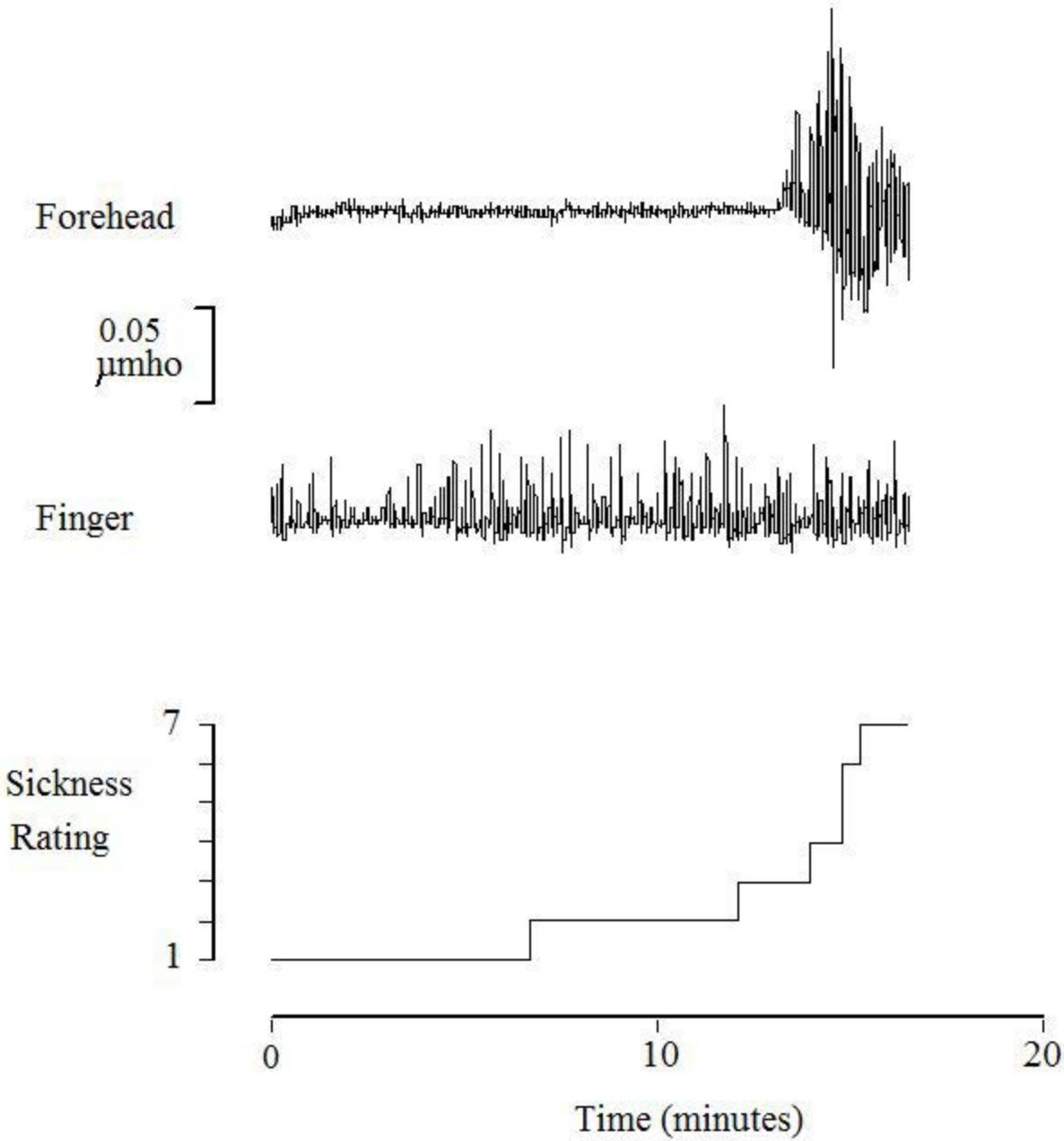
Figure 4. – Palmar finger skin conductance activity plots for one subject over the four treatments, prior to the onset of motion sickness. All traces are of 4 minutes duration starting at the beginning of the motion challenge, time zero. Note that oral Hyoscine hydrobromide (HBr) 0.6 mg and oral Darifenacin 10 mg and 20 mg, all reduce skin conductance activity by comparison with Placebo. The effect was most marked for the high Darifenacin dose.

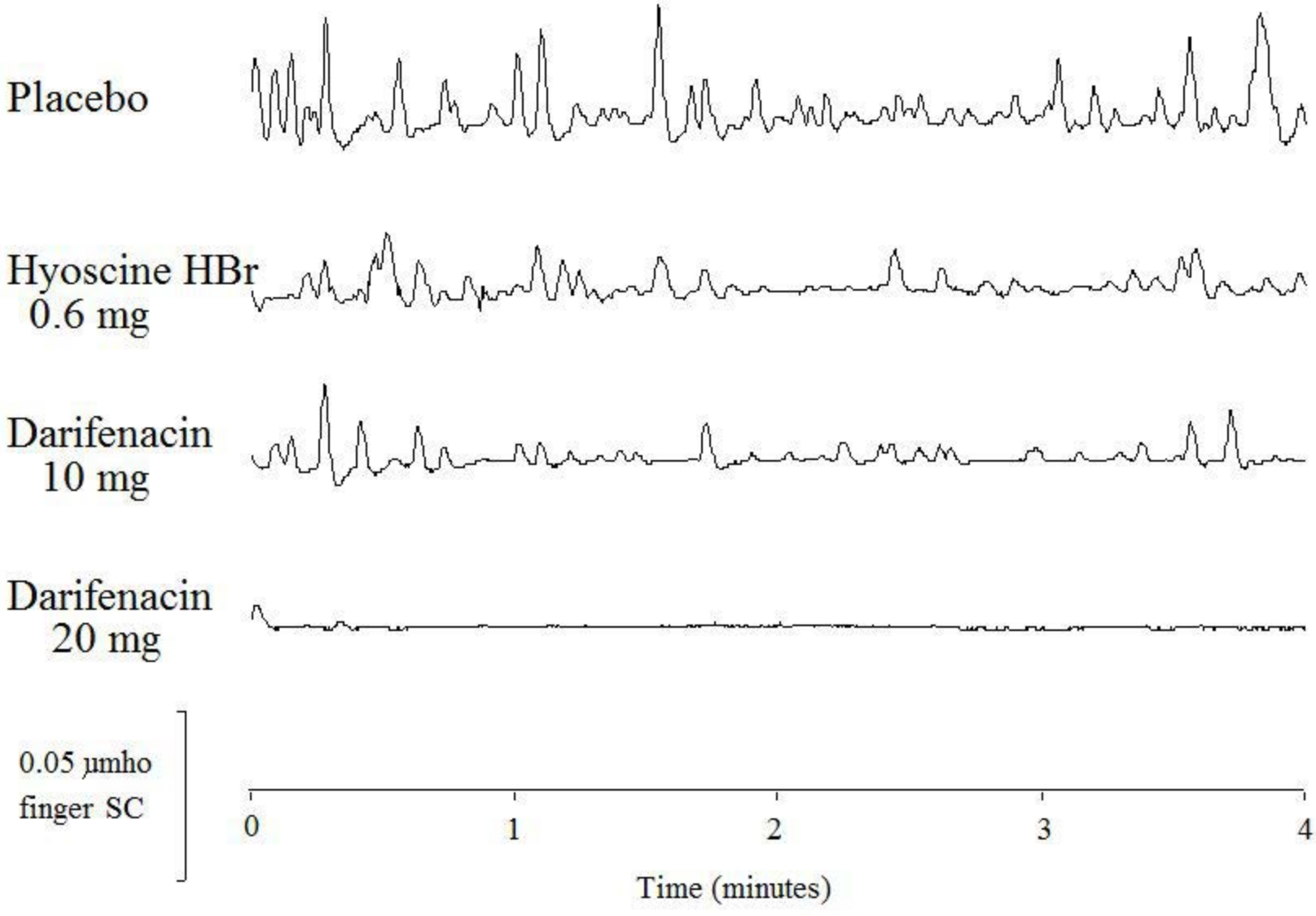
Figure 5. - Mean phasic skin conductance activity, at the palmar finger and forehead sites, is plotted for the four treatment conditions. The mean phasic skin conductance activity is based on two minute samples at the beginning of the motion challenge prior to motion sickness, and two minute samples at the end of the motion challenge during maximum motion sickness. Darifenacin and Hyoscine hydrobromide (HBr) significantly reduced phasic skin conductance activity compared with Placebo (for example see Figure 3). At the forehead recording site, the rise in skin conductance activity over time represented motion sickness induced sweating (for example see forehead signal in Figure 4).

Supplementary Figures 1-3. – Motion sickness test on rotating chair showing head movements.


Mean Time (min) to Motion Endpoint
25
24
23
22
21
20
19
18
17
16
*
**
**
No exclusions
Exclude 1 session Hyoscine
Maximum exclusions: 5 sessions
Placebo
Hyoscine HBr 0.6 mg
Darifenacin 10mg
Darifenacin 20mg
Error bars: +/- 1 SE
* p<0.05
** p<0.01
Drug vs Placebo


*** p<0.001 * p<0.05
Drug vs Placebo
Placebo
Hyoscine HBr 0.6mg
Darifenacin 10mg
Darifenacin 20mg
Error bars: +/- 1 SE
Mean Symptom Rating
3.5
3.0
2.5
2.0
1.5
1.0
.5
.0
Nausea
Stomach
Dizziness
Warmth
Salivation
Taste
Dry Mouth
Headache
*
*
*
*
***


Forehead
0.05
µmho
Finger
Sickness
Rating
7
1
0
10
20
Time (minutes)


Placebo
Hyoscine HBr
0.6 mg
Darifenacin
10 mg
Darifenacin
20 mg
0.05 µmho
finger SC
0
1
2
3
4
Time (minutes)

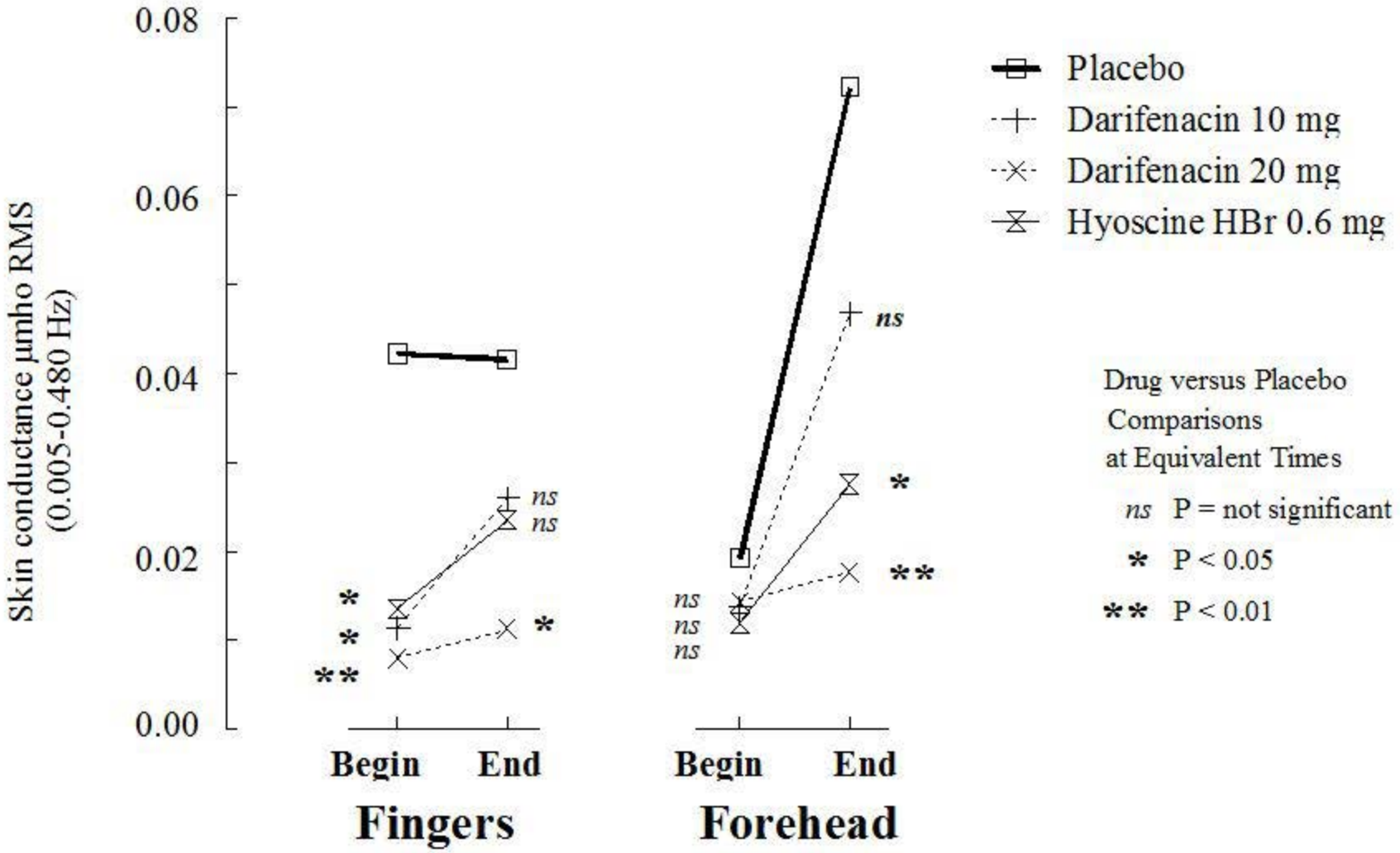

Skin conductance µmho RMS
(0.005-0.480 Hz)
0.08
0.06
0.04
0.02
0.00
Placebo
Darifenacin 10 mg
Darifenacin 20 mg
Hyoscine HBr 0.6 mg
ns
ns
*
*
**
*
Begin
End
Fingers
ns
ns
ns
ns
*
**
Begin
End
Forehead
Drug versus Placebo
Comparisons
at Equivalent Times
ns P = not significant
* P < 0.05
** P < 0.01

Table 1. Mean times minutes to motion endpoint

Descriptive Statistics

	Mean	Standard Deviation	N
Practice	14.63	5.22	15
Placebo	18.30	5.73	15
Hyoscine hydrobromide 0.6 mg	20.70	7.70	15
Darifenacin 10mg	18.93	7.46	15
Darifenacin 20mg	18.90	7.31	15

Supplementary Table 1. Psychomotor and cognitive performance tests: mean (SD) pre- and post-drug (control: Placebo; Dar10: Darifenacin 10 mg; Dar20 Darifenacin 20 mg; Hyoscine: Hyoscine hydrobromide 0.6 mg), oral drug was given at time zero.

Test (n = 15)	Drug Type	Time (Hours)			
		-1	1.5	3	4.5
Simple Reaction Time (msec)	Control	232.4 (20.27)	262.7 (52.24)	262.7 (60.70)	259.9 (49.82)
	Dar10	250.3 (34.23)	255.1 (30.28)	268.2 (41.83)	260.2 (43.25)
	Dar20	243.1 (45.60)	249.5 (37.98)	252.5 (36.48)	261.8 (47.97)
	Hyoscine	242.9 (22.36)	268.5 (31.67)	269.0 (41.56)	262.9 (35.15)
Digit Vigilance –Correct Detect (%)	Control	98.96 (2.03)	98.96 (2.50)	98.37 (1.96)	98.37 (1.56)
	Dar10	98.08 (2.50)	99.11 (1.84)	96.89 (3.83)	97.93 (2.58)
	Dar20	97.78 (3.03)	98.07 (4.59)	98.07 (2.36)	97.78 (3.36)
	Hyoscine	99.11 (2.02)	97.04 (3.53)	97.63 (2.58)	96.74 (3.01)
Digit Vigilance – Speed (msec)	Control	404.2 (35.70)	415.6 (38.53)	415.4 (39.42)	418.6 (41.42)
	Dar10	420.8 (45.77)	416.0 (51.29)	426.3 (49.38)	423.8 (40.72)
	Dar20	408.1 (39.46)	424.3 (47.64)	420.8 (46.98)	424.0 (47.25)
	Hyoscine	419.5 (41.98)	422.6 (47.76)	424.7 (42.61)	427.9 (37.14)
Digit Vigilance –False Alarms	Control	0.733 (0.96)	1.000 (1.41)	0.867 (1.06)	0.533 (1.06)
	Dar10	0.933 (1.10)	0.267 (0.59)	0.333 (0.62)	0.333 (0.82)
	Dar20	0.933 (1.28)	0.733 (0.80)	1.067 (2.02)	0.600 (1.35)
	Hyoscine	0.867 (1.13)	0.400 (0.74)	0.600 (0.99)	1.133 (2.13)
Choice Reaction Time –Accuracy (%)	Control	94.67 (6.79)	96.67 (2.90)	96.00 (4.00)	93.87 (6.35)
	Dar10	95.47 (4.98)	95.33 (5.88)	96.00 (2.51)	94.53 (3.96)
	Dar20	96.13 (3.96)	96.13 (5.37)	93.33 (5.33)	95.33 (5.43)
	Hyoscine	97.20 (1.97)	94.27 (6.36)	95.33 (5.12)	92.80 (8.02)
Choice Reaction Time: Speed (msec)	Control	403.7 (57.84)	415.0 (62.68)	414.4 (72.36)	414.3 (76.37)
	Dar10	413.0 (70.27)	406.7 (60.58)	425.3 (74.52)	417.1 (61.98)
	Dar20	414.2 (49.98)	408.3 (57.56)	403.1 (50.93)	406.7 (69.04)
	Hyoscine	414.2 (64.64)	414.2 (60.60)	420.2 (62.53)	415.9 (58.89)
Tracking Mean Error (mm)	Control	22.93 (3.21)	23.35 (3.73)	23.10 (2.90)	23.68 (3.83)
	Dar10	23.06 (2.81)	23.59 (4.37)	22.86 (3.41)	23.47 (4.64)
	Dar20	23.41 (3.42)	23.11 (3.30)	23.33 (3.37)	23.09 (3.56)
	Hyoscine	22.44 (3.48)	23.77 (3.96)	23.87 (3.43)	25.67 (9.19)
Numeric Working Memory – Sensitivity	Control	0.894 (0.10)	0.867 (0.13)	0.857 (0.15)	0.842 (0.14)
	Dar10	0.883 (0.07)	0.889 (0.09)	0.911 (0.06)	0.904 (0.08)
	Dar20	0.908 (0.07)	0.888 (0.09)	0.878 (0.10)	0.856 (0.13)
	Hyoscine	0.906 (0.07)	0.880 (0.10)	0.876 (0.13)	0.846 (0.14)
Numeric Working Memory – Speed	Control	568.2 (81.95)	565.9 (87.70)	559.2 (73.39)	542.9 (70.62)
	Dar10	556.7 (75.43)	558.8 (71.00)	550.7 (78.99)	553.6 (77.52)
	Dar20	565.0 (62.73)	559.2 (72.42)	548.8 (62.06)	553.8 (70.17)
	Hyoscine	575.1 (93.16)	576.8 (79.88)	563.3 (80.43)	579.9 (99.23)
Word Recognition – Sensitivity Index	Control	0.674 (0.19)	0.561 (0.20)	0.533 (0.23)	0.504 (0.31)
	Dar10	0.678 (0.18)	0.560 (0.20)	0.612 (0.18)	0.533 (0.13)
	Dar20	0.591 (0.18)	0.532 (0.22)	0.523 (0.23)	0.485 (0.22)
	Hyoscine	0.608 (0.19)	0.528 (0.27)	0.538 (0.19)	0.431 (0.36)
Word Recognition – Speed (msec)	Control	655.5 (133.7)	677.0 (118.1)	701.1 (136.9)	642.7 (111.2)
	Dar10	670.0 (90.26)	668.9 (118.6)	704.3 (107.9)	690.5 (144.8)
	Dar20	653.3 (79.29)	661.4 (111.7)	676.8 (115.4)	720.3 (150.6)
	Hyoscine	711.5 (93.38)	649.7 (113.7)	709.2 (149.5)	682.2 (157.3)
Critical Flicker Fusion (HE)	Control	41.07 (4.11)	41.57 (4.20)	41.67 (3.75)	42.53 (3.20)
	Dar10	41.73 (3.04)	41.87 (3.54)	41.87 (4.29)	41.60 (3.23)
	Dar20	40.67 (4.00)	42.07 (3.58)	41.93 (4.25)	42.53 (3.76)
	Hyoscine	41.20 (3.32)	41.13 (2.95)	41.73 (3.56)	41.27 (5.35)

Test (n = 15)	Drug Type	Time (Hours)			
		-1	1.5	3	4.5
Body Sway	Control	21.33 (2.81)	17.80 (12.04)	19.27 (3.17)	16.33 (9.93)
	Dar10	17.40 (9.13)	15.67 (7.87)	17.80 (11.52)	19.40 (12.13)
	Dar20	15.33 (6.33)	15.93 (7.67)	16.80 (11.09)	18.07 (12.27)
	Hyoscine	16.13 (9.55)	20.60 (12.98)	20.53 (19.18)	20.40 (15.06)
Digit Symbol Test (n correct)	Control	76.47 (13.49)	76.93 (14.38)	75.80 (14.56)	77.20 (14.23)
	Dar10	73.87 (13.50)	76.13 (13.05)	75.40 (13.87)	76.07 (12.75)
	Dar20	74.80 (11.83)	75.80 (12.32)	75.87 (12.84)	75.93 (12.12)
	Hyoscine	76.60 (12.97)	76.33 (14.04)	76.73 (13.51)	75.07 (15.28)
Immediate Word Recall Accuracy (%)	Control	54.66 (16.80)	45.77 (14.34)	45.55 (14.46)	44.66 (13.89)
	Dar10	48.88 (14.18)	48.11 (13.78)	45.89 (11.93)	45.22 (13.23)
	Dar20	50.11 (9.22))	46.33 (12.79)	46.33 (11.48)	42.55 (11.56)
	Hyoscine	48.44 (15.10)	43.77 (17.87)	48.00 (12.07)	41.44 (10.78)
Delayed Word Recall Accuracy %	Control	39.77 (19.62)	27.00 (11.87)	25.44 (15.07)	22.44 (13.60)
	Dar10	34.22 (12.05)	26.88 (11.85)	21.33 (12.65)	22.00 (8.34)
	Dar20	32.00 (13.20)	29.55 (13.43)	21.11 (10.42)	19.77 (11.02)
	Hyoscine	30.55 (11.54)	23.44 (15.59)	20.66 (12.10)	20.44 (14.41)
Alertness	Control	65.63 (11.2)	60.69 (13.00)	54.87 (15.42)	57.39 (16.51)
	Dar10	58.21 (17.76)	65.47 (15.73)	59.06 (15.70)	58.03 (17.21)
	Dar20	62.13 (18.69)	60.12 (12.93)	59.48 (16.45)	57.54 (14.92)
	Hyoscine	59.55 (13.54)	51.57 (17.47)	49.82 (17.16)	52.51 (15.21)
Contentment	Control	76.57 (13.78)	76.56 (14.01)	71.51 (12.85)	74.29 (17.92)
	Dar10	75.92 (12.83)	75.73 (14.06)	78.44 (12.70)	76.00 (13.67)
	Dar20	73.05 (16.03)	75.51 (14.80)	70.87 (16.07)	71.53 (14.38)
	Hyoscine	76.04 (9.90)	74.36 (12.59)	73.77 (14.13)	73.99 (13.49)
Calmness	Control	70.90(13.66)	70.27 (11.81)	71.83 (10.63)	73.67 (11.97
	Dar10	73.07 (11.01)	75.33 (11.91)	76.30 (12.60)	74.90 (11.89)
	Dar20	72.97 (14.96)	73.47 (11.92)	72.87 (13.43)	67.00 (17.45)
	Hyoscine	71.07 (8.83)	71.87 (11.57)	75.47 (10.13)	75.43 (7.17)

Test (n = 15)	Drug Type	Time (Hours)		
		DB1	DB3	DB4
Alertness	Control	-4.940 (11.46)	-10.75 (12.16)	-8.240 (14.03)
	Dar10	7.253 (16.58)	0.847 (13.22)	-0.187 (14.79)
	Dar20	-2.007 (16.43)	-2.647 (16.74)	-4.587 (18.16)
	Hyoscine	-7.973 (13.19)	-9.727 (14.16)	-7.033 (15.79)
Calmness	Control	-0.633 (11.15)	0.933 (13.85)	2.767 (11.33)
	Dar10	2.267 (17.33)	3.233 (13.05)	1.833 (15.61)
	Dar20	0.500 (8.045)	-0.100 (16.57)	-5.967 (21.78)
	Hyoscine	0.800 (14.30)	4.400 (12.50)	4.367 (10.21)
Critical flicker fusion frequency	Control	0.786 (2.01)	0.600 (2.23)	1.467 (2.20)
	Dar10	0.133 (1.31)	0.133 (2.07)	-0.133 (1.18)
	Dar20	1.400 (3.57)	1.267 (2.15)	1.867 (2.42)
	Hyoscine	-0.067 (1.71)	0.533 (1.51)	0.0667 (2.74)
Contentment	Control	-0.013 (6.05)	-5.067 (8.77)	-2.280 (12.51)
	Dar10	-0.187 (10.03)	2.520 (7.55)	0.08 (13.20)
	Dar20	2.453 (8.98)	-2.187 (11.61)	-1.520 (13.00)
	Hyoscine	-1.680 (6.38)	-2.267 (8.53)	-2.053 (5.85)
Choice reaction time	Control	11.34 (22.56)	10.73 (28.21)	10.59 (33.68)
	Dar10	-6.245 (24.56)	12.33 (24.19)	4.140 (25.89)
	Dar20	-5.913 (27.41)	-11.12 (19.58)	-7.557 (35.81)
	Hyoscine	0.054 (42.01)	6.024 (41.38)	1.723 (32.83)
Choice reaction time – Accuracy	Control	2.000 (7.64)	1.333 (6.66)	-0.800 (6.67)
	Dar10	-0.133 (3.50)	0.533 (3.66)	-0.933 (4.13)
	Dar20	0 (3.67)	-2.800 (4.88)	-0.800 (3.10)
	Hyoscine	-2.933 (5.65)	-1.867 (4.31)	-4.400 (7.38)
Delayed word recall – Accuracy	Control	-12.78 (20.30)	-14.33 (18.12)	-17.33 (25.58)
	Dar10	-7.335 (14.92)	-12.89 (14.79)	-12.22 (10.37)
	Dar20	-2.443 (8.47)	-10.89 (13.76)	-12.22 (21.11)
	Hyoscine	-7.112 (16.43)	-9.890 (14.40)	-10.11 (15.18)
DRECRT	Control	21.50 (65.89)	45.64 (69.62)	-12.78 (80.86)
	Dar10	-1.099 (69.45)	34.33 (64.56)	20.49 (89.40)
	Dar20	8.069 (75.10)	23.44 (113.5)	66.94 (119.3)
	Hyoscine	-61.71 (94.81)	-2.235 (110.3)	-29.29 (120.9)
Word recognition – Sensitivity index	Control	-0.113 (0.20)	-0.141 (0.17)	-0.170 (0.25)
	Dar10	-0.118 (0.20)	-0.065 (0.22)	-0.145 (0.21)
	Dar20	-0.059 (0.18)	-0.068 (0.20)	-0.106 (0.17)
	Hyoscine	-0.08 (0.27)	-0.069 (0.25)	-0.177 (0.34)
Digit symbol test	Control	0.467 (2.48)	-0.667 (4.29)	0.733 (4.37)
	Dar10	2.267 (5.47)	1.533 (5.49)	2.200 (5.74)
	Dar20	1.000 (3.76)	1.067 (5.50)	1.133 (4.37)
	Hyoscine	-0.267 (3.96)	0.133 (4.05)	-1.533 (5.08)
Immediate word recall – Accuracy	Control	-8.889 (15.28)	-9.111 (13.06)	-9.999 (14.73)
	Dar10	-0.777 (11.39)	-2.999 (10.77)	-3.665 (12.25)
	Dar20	-3.777 (11.35)	-3.777 (8.273)	-7.554 (15.42)
	Hyoscine	-4.667 (11.36)	-0.444 (10.77)	-7.001 (14.90)
MSRT	Control	-2.309 (37.73)	-8.949 (35.36)	-25.24 (42.49)
	Dar10	2.184 (46.93)	-5.934 (35.26)	-3.073 (38.05)
	Dar20	-5.866 (39.78)	-16.24 (32.73))	-11.24 (50.42)
	Hyoscine	1.640 (34.32)	-11.83 (44.15)	4.759 (70.59)
MSSI	Control	-0.027 (0.07)	-0.037 (0.08)	-0.053(0.09)
	Dar10	0.0056 (0.09)	0.0282 (0.07)	0.0211 (0.08)
	Dar20	-0.02 (0.07)	-0.03 (0.08)	-0.052 (0.13)
	Hyoscine	-0.027 (0.07)	-0.03 (0.10)	-0.06 (0.12)
Simple reaction time (msec)	Control	30.31 (37.35)	30.34 (46.08)	27.55 (32.47)
	Dar10	4.845 (21.33)	17.87 (17.91)	9.953 (28.77)
	Dar20	6.379 (20.56)	9.335 (28.18)	18.67 (26.98)
	Hyoscine	25.65 (16.81)	26.08 (26.74)	20.07 (24.04)

Test (n = 15)	Drug Type	Time (Hours)		
		DB1	DB3	DB4
Body sway	Control	-3.533 (8.43)	-2.067 (8.79)	-5.000 (5.79)
	Dar10	-1.733 (8.16)	0.400 (7.81)	2.000 (6.50)
	Dar20	0.600 (4.718)	1.467 (10.08)	2.733 (10.24)
	Hyoscine	4.467 (6.37)	4.400 (13.47)	4.267 (10.63)
Tracking mean error	Control	0.425 (2.02)	0.336 (1.30)	0.756 (2.01)
	Dar10	0.532 (3.18)	-0.199 (1.99)	0.414 (3.44)
	Dar20	-0.293 (2.25)	-0.08 (1.93)	-0.314 (2.11)
	Hyoscine	1.328 (2.27)	1.428 (1.71)	3.230 (7.46)
Digit vigilance – Accuracy	Control	0 (3.46)	-0.592 (2.84)	-0.591 (2.96)
	Dar10	1.036 (3.01)	-1.185 (4.19)	-0.148 (2.84)
	Dar20	0.296 (3.24)	0.296 (3.35)	-67E-5 (3.85)
	Hyoscine	-2.072 (4.07)	-1.481 (2.74)	-2.370 (2.72)
Digit vigilance – False alarm	Control	0.267 (1.58)	0.133 (0.99)	-0.200 (1.42)
	Dar10	-0.667 (0.82)	-0.600 (1.24)	-0.600 (0.83)
	Dar20	-0.200 (1.32)	0.133 (2.39)	-0.333 (2.02)
	Hyoscine	-0.467 (1.25)	-0.267 (1.34)	0.267 (2.40)
Digit vigilance – Reaction time	Control	11.44 (20.35)	11.25 (28.34)	14.40(33.23)
	Dar10	-4.799 (26.46)	5.453 (29.67)	2.974(26.75)
	Dar20	16.13 (28.00)	12.71 (19.39)	15.91(27.44)
	Hyoscine	3.145 (31.63)	5.209 (33.51)	8.456 (29.35)

Supplementary Table 2. Times (Minutes) To A Sickness Rating of 7

Subject	Practice	Placebo	Hyoscine	Darifenacin 10mg	Darifenacin 20mg
1	14.5	15.5	18.0	14.0	17.0
2	15.5	14.5	18.0	17.5	17.0
3	21.0	27.0	30.0	20.5	23.5
4	19.5	20.0	25.0	20.0	21.5
5	9.0	21.5	8.5	19.5	21.0
6	13.0	17.0	18.0	19.0	15.5
7	10.5	12.5	13.5	11.0	12.0
8	13.5	15.0	18.0	16.0	14.0
9	25.0	26.5	N/A	N/A	N/A
10	26.5	31.5	29.5	41.0	39.0
11	10.5	14.5	13.0	12.5	13.0
12	7.0	15.5	18.5	14.0	18.0
13	14.0	18.0	22.5	21.5	15.0
14	20.5	25.5	39.0	28.0	29.5
15	11.5	14.5	22.0	15.0	15.5
16	13.0	12.0	17.0	14.5	12.0

Subject 4 was excluded in the Darifenacin 20mg session.

Subject 5 was excluded in the Hyoscine session.

Subject 9 did not complete the study.

Subject 12 was excluded in the Darifenacin 10mg session.

Subject 14 was excluded in the Darifenacin 10mg session.

These subjects were excluded based on reports of no nausea at the motion endpoint.

Supplementary Table 3: Molecular and pharmacological properties of antimuscarinic compounds

Antagonist		**Darifenacin**	**Oxybutynin**	**Solifenacin**	**Tolterodine**	**Hyoscine**	**Zamifenacin**
Physicochemical properties [30]	Molecular weight	507.5	357 (chloride - 393.9[b])	480.55	475.6	303.356 (hydrobromide - 384.27)	415.533 (fumerate - 531.605)
	Polarity	Positive	Neutral	Neutral	Positive	Neutral	Neutral
	Lipophilicity	High	Moderate	Moderate	Low	Low	Low
	BBB penetration potential	Moderate/High (but bladder selective & P-gp substrate)	High	High	Moderate/High	High	N/A
	Bioavailability (%)	15-19	6	90	77	10-50	N/A
	Protein binding (%)	98	N/A	98	96	11	N/A
	Terminal elimination half-life (h)	7-20	13	45-68	2-10	4.5	N/A
	Metabolite	None	Desethyl-oxybutynin	None	5-HMT	None	N/A
	P-glycoprotein substrate	Yes	No	No	No	No	N/A
Receptor subtype selectivity (pK_i)	M1	8.2 (0.04) [35]	8.7 (0.04) [35]	7.6	8.8 (0.01) [35]	8.95 (0.31) [39]	7.2 [37]
	M2	7.4 (0.10) [35]	7.8 (0.10) [35]	7.1	8.0 (0.10) [35]	8.68 (0.08) [39]	7.9 [37]
	M3	9.1 (0.10) [35]	8.9 (0.10) [35]	7.7	8.5 (0.10) [35]	9.41 (0.07) [39]	6.9 [37]
	M4	7.3 (0.10) [35]	8.0 (0.04) [35]	6.8	8.0 (0.04) [35]	9.47 (0.06) [39]	7.3 [38]
	M5	8.0 (0.10) [35]	7.4 (0.03) [35]	7.2	7.4 (0.03) [35]	N/A	N/A
Receptor affinity ratio [35]	M3:M1	9.3**	1.5*	2.5	0.6*	N/A	N/A
	M3:M2	59.2**	12.3**	N/A	3.6**	N/A	N/A
Effect on cognition		No significant change (works in combination with AChEI)	Significant impairment[30]	No significant change[30]	No significant change[30]	Significant impairment[7]	N/A
Motion sickness effect		Mild negative[7]	N/A	N/A	N/A	High positive[8]	Moderate positive [8]

N/A – not available

a Ki ratios were compared by ANOVA. *p<0.05, **p<0.001 [35]

b drug administered as oxybutynin chloride

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