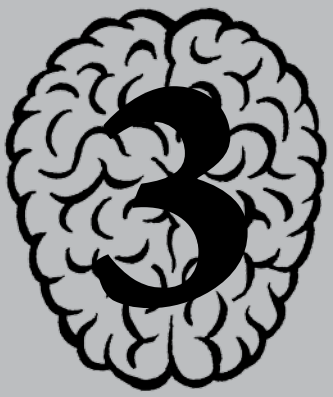
Challenging the cholinergic system : ageing, cognition & inflammation

Alvarez-Jiménez, R.



AN ANTI-NICOTINIC COGNITIVE CHALLENGE MODEL USING MECAMYLAMINE IN COMPARISON WITH THE ANTI-MUSCARINIC COGNITIVE CHALLENGE USING SCOPOLAMINE

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A.C. Baakman*, R. Alvarez-Jimenez*,
R. Rissmann, E.S. Klaassen, J. Stevens,
S.C. Goulooze, J. Burger, E.L. Swart,
J.M.A. van Gerven, G.J. Groeneveld
** Both authors contributed equally*

ABSTRACT

The muscarinic acetylcholine receptor antagonist scopolamine is often used for proof-of-pharmacology studies with pro-cognitive compounds. From a pharmacological point of view, it would seem more rational to use a nicotinic rather than a muscarinic anticholinergic challenge to prove pharmacology of a nicotinic acetylcholine receptor agonist. This study aims to characterize a nicotinic anticholinergic challenge model using mecamylamine and to compare it to the scopolamine model. In this double blind, placebo controlled, four way cross-over trial 12 healthy male subjects received oral mecamylamine 10 and 20 mg, intravenous scopolamine hydrobromide 0.5 mg and placebo. Pharmacokinetics were explored using non-compartmental analysis. Pharmacodynamic effects were measured with a multidimensional test battery that includes neurophysiological, subjective, (visuo)motor and cognitive measurements. All treatments were safe and well tolerated. Mecamylamine had a T_{max} of 2.5 hours and a C_{max} of 64.5 ng·ml^{-1} for the 20 mg dose. Mecamylamine had a dose dependent effect which decreased the adaptive tracking performance, VAS alertness, finger tapping time and performance in the visual verbal learning task. No effects were seen on the simple reaction time test or saccadic peak velocity. Scopolamine significantly affected almost all pharmacodynamic tests. This study demonstrated that mecamylamine causes nicotinic receptor specific temporary decline in cognitive functioning. Compared with the scopolamine model, pharmacodynamic effects were less pronounced at the dose levels tested, but mecamylamine caused less sedation. The cognitive effects of scopolamine might at least partly be caused by sedation. Whether the mecamylamine model can be used for proof-of-pharmacology of nicotinic acetylcholine receptor agonists remains to be established.

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia, with a prevalence of 3–7% in the Western European population (Takizawa *et al*, 2015). AD causes significant burden for the patients and their caregivers and high health care costs for society. Even though many research groups aim to unravel the pathophysiology and many pharmaceutical companies are searching for pharmacological targets for a curative treatment, no new drugs have been registered for this indication since 2003. The only approved therapy for mild to moderate AD is symptomatic treatment with cholinesterase inhibitors (CEIs), increasing the acetylcholine level in the synaptic cleft of cholinergic neurons. The cholinergic system is hypothesized to play an important role in several cognitive processes such as attention and memory (Drachman and Leavitt, 1974). Also, pathology studies have shown decreased levels of acetylcholine levels in the brains of patients with AD. Nevertheless, treatment with CEIs is only effective in about 14–36% of the AD patients and the dose is limited by peripheral side effects such as nausea, vomiting and diarrhoea (Birks, 2006; Birks *et al*, 2009; Olin and Schneider, 2002; Rösler *et al*, 1999; Tariot *et al*, 2000). CEIs inhibit esterases peripherally and in the central nervous system (CNS) so they will not only enhance functioning of cholinergic neuronal system, but will also induce peripheral cholinergic side effects, mainly via autonomic parasympathetic neurons. These peripheral side effects could be avoided with agonists that are more selective for AChRs with a higher presence in the CNS than peripherally, such as the α_7 and $\alpha_4\beta_2$ nicotinic acetylcholine receptor (nAChR). nAChR are mainly located in the hippocampus, thalamus, amygdala, striatum, entorhinal, frontal and pre-frontal cortex. Based on the localization of nAChR in the human brain, nicotinergic blockade could be expected to result in an impairment of cognitive functions such as acquisition, processing and recall of information (Paterson and Nordberg, 2000). Accumulating evidence suggests that α_7 nAChRs play an important role in the pathophysiology of neuropsychiatric diseases, including schizophrenia and AD. Hence, a number of pharmaceutical industries have developed selective and high affinity α_7 nAChR agonists as therapeutic drugs for these neuropsychiatric diseases

(Toyohara and Hashimoto, 2010). Therefore, specific agonists targeting nAChR are currently being developed.

Proof-of-pharmacology studies with cholinergic compounds are often performed in healthy subjects after administration of scopolamine (Blin *et al*, 2009; Buccafusco, 2009; Cho *et al*, 2011; Deiana *et al*, 2009; Lee *et al*, 2009; Liem-Moolenaar *et al*, 2010; van Ruitenbeek *et al*, 2008; Snyder *et al*, 2005). Scopolamine is a competitive muscarinic acetylcholine receptor (mAChR) antagonist with similar binding to all five known muscarinic receptor subtypes. From a pharmacological point of view, it seems more rational to use a nicotinic rather than a muscarinic anticholinergic challenge in a proof of pharmacology study of a nicotinic acetylcholine receptor agonist.

Mecamylamine is a nAChR antagonist that has been used for the treatment of severe hypertension since the 1950s. In 2009 it was withdrawn from the market because of its unfavourable risk-benefit profile compared with many other available antihypertensives. Mecamylamine's antihypertensive effects are mediated through nAChR in peripheral autonomic ganglia. However, it also binds to nAChR present in the CNS (Stone *et al*, 1956). Previous studies have confirmed that mecamylamine, temporarily and reversibly, perturbs the above-mentioned cognitive processes in healthy volunteers (Little *et al*, 1998; Newhouse *et al*, 1992, 1994; Thompson *et al*, 2000; Voss *et al*, 2010).

With this study we aimed to better characterize the pharmacodynamic and pharmacokinetic effects of mecamylamine compared to scopolamine in order to improve the knowledge about a nAChR specific anti-cholinergic challenge and to develop a challenge model that may be suitable for proof-of-pharmacology studies with nAChR agonists.

METHODS

TRIAL DESIGN AND SUBJECTS

This double blind, double dummy, placebo controlled, four-way cross-over study was performed in healthy, non-smoker, young male subjects. On four different occasions with a wash-out of 7 days in between, all subjects received an oral dose of mecamylamine 10 mg with intravenous placebo, an oral dose of mecamylamine 20 mg with intravenous placebo, an intravenous dose of scopolamine hydrobromide 0.5 mg with oral placebo and both oral and intravenous placebo. The expected T_{max} of scopolamine was 15 minutes after the start of the infusion, while the expected T_{max} of mecamylamine was 3 hours after oral administration(Liem-Moolenaar *et al*, 2011; Young *et al*, 2001). Therefore, the intravenous dose of scopolamine or placebo was given 2.45 hours after administration of mecamylamine or placebo with infusion duration of 15 minutes in order to have a T_{max} of both drugs at approximately the same time point. All subjects gave written informed consent for participation in the study. The ethics committee of the Leiden University Medical Center (The Netherlands) approved the study.

DOSING RATIONALE

For the treatment of hypertension, the approved starting dose of mecamylamine was 25 mg per day and in various cognitive studies, a maximum of 20 mg orally produced few adverse effects, other than mild hypotension (Dumas *et al*, 2006, 2008, 2010; Ellis *et al*, 2006; Erskine *et al*, 2004; Ford *et al*, 1956; Green *et al*, 2005; Little *et al*, 1998; Newhouse *et al*, 1992, 1994; Thienel *et al*, 2009; Thompson *et al*, 2000; Voss *et al*, 2010; Young *et al*, 2001). Cognitive impairments are observed at dose levels of 15 mg and higher (Little *et al*, 1998; Newhouse *et al*, 1992, 1994; Thompson *et al*, 2000). For the pharmacological challenge in this study a lower (10 mg) and higher (20 mg) dose were chosen in order to better determine concentration-effect relationships. Mecamylamine uptake is characterized by complete absorption from the gastrointestinal tract (Young *et al*, 2001).

Scopolamine has been validated and frequently used as a pharmacological challenge in previously published studies with minimal adverse effects and demonstrable cognitive impairments at 0.5 mg scopolamine intravenously dosed (Liem-Moolenaar *et al*, 2011).

PHARMACOKINETICS

Venous blood samples were obtained via an indwelling catheter before administration of mecamylamine or placebo and at 0.5, 1.0, 2.0, 3.0, 3.25, 4.0,

6.0, 8.0, 10.0 and 22.0 hours after drug administration. Plasma concentrations of mecamylamine and scopolamine were determined at the department of Clinical Pharmacology and Pharmacy at the VU University Medical Centre (Amsterdam, The Netherlands) by a validated method using high performance liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS).

The LC-MS/MS consisted of a Waters Alliance 2795 separation module and a Quattro Micro tandem mass spectrometer from Waters (Watford, UK). System control, data acquisition and data processing were performed using MassLynx v4.1. Chromatography was performed on a Kinetex C18 analytical column from Phenomenex. The particle size was 2.6 µM, column length was 150 mm and column diameter was 3.0 mm. The mobile phase ratio of 70% mobile phase A and 30% mobile phase B was run with a flow of 0.5 $mL \cdot min^{-1}$. Both mobile phases contained 0.05 % (v/v) trifluoretic acid and 5 mM ammoniumformate, whereas mobile phase A was prepared in purified water and mobile phase B was prepared in methanol. Ionization of the drugs was achieved in the positive electrospray modus. The respective MRM transitions were 168.1 > 137.1 m/z for mecamylamine, 304.2 > 138.1 m/z for scopolamine, 171.2 > 137.1 m/z for mecamylamine-D3 and 307.1 > 141.1 m/z for scopolamine-D3. For sample preparation, 100 µL of an aqueous solution containing 1 M zinc sulphate was added to 40 µL plasma and short vortexed. Hereafter 100 µL of the internal standard was added containing 100 $\mu g \cdot L^{-1}$ of mecamylamine-D3 and scopolamine-D3 in methanol. After vortexing for 3 minutes the samples were centrifuged at 10900 g for 3 minutes. The clear supernatant was transferred to vials and 25 µL was injected on the LC-MS/MS.

PHARMACODYNAMIC ASSESSMENTS

To determine the pharmacodynamic effects of mecamylamine, a battery of tests (NeuroCart®) with a previously shown sensitivity to drug effects on a wide range of CNS domains was used (Liem-Moolenaar *et al*, 2011; van Steveninck *et al*, 1991, 1999; de Visser *et al*, 2003). All tests were performed twice at baseline, and repeated at 1.0, 2.0, 3.25, 4.0, 6.0, 8.0 and 10.0 hours after administration of mecamylamine or placebo. The only exception was the visual verbal learning test, which was performed 3.5 hours after dosing (immediate recall) and 5 hours after dosing (delayed recall and recognition). Measurements were performed in a quiet room with ambient illumination with only one subject per session in the same room.

FINGER TAPPING ✳ This test evaluates motor activation and fluency and has been adapted from the Halstead Reitan Test Battery (Andrew, 1977). The volunteer was instructed to tap as quickly as possible with the index finger of the dominant hand. Each session contained 5 performances of 10 seconds. Feedback on performance was given by a counter in the centre of the screen, while the amount of taps of each 10 second trial was shown on the screen in between the trials. The mean tapping rate of five trials per time point was used for statistical analysis.

N-BACK ✳ This test evaluates the working memory and requires buffering and updating consonants, matching, encoding and responding. The N-back test consists of three conditions, with increased working memory load. Letters were presented consecutively on the screen with a speed of 30 letters per minute. In the first condition subjects had to indicate whether the letter on the screen was an 'X'. In the second condition, subjects indicated whether the letter seen was identical to the previous letter. In the third condition, subjects were asked to indicate whether the letter was identical to two letters before the letter seen (Lim *et al*, 2008; Rombouts *et al*, 2002; Sweet *et al*, 2006).

ADAPTIVE TRACKING ✳ Adaptive tracking is a pursuit-tracking task, measuring attention and eye-hand coordination. A circle moves pseudo-randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance scores over a three-minute period was used for analysis. Before study participation, subjects performed three training sessions and at each occasion two baseline measurements were done (Gijsman *et al*, 1998; van Steveninck *et al*, 1991, 1993, 1999).

SACCADIC PEAK VELOCITY ✳ Saccadic peak velocity (SPV) is one of the most sensitive parameters for sedation. The use of a computer for measurement of saccadic eye movements has been described elsewhere (Baloh *et al*, 1975; van Steveninck *et al*, 1991, 1999). Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

SMOOTH PURSUIT EYE MOVEMENTS ✳ The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moves at a frequency ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as parameter (Baloh *et al*, 1975; Bittencourt *et al*, 1983).

PHARMACO-ELECTROENCEPHALOGRAPHY ✳ Pharmacoelectroencephalography (p-EEG) was used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain (Cohen *et al*, 1985; Van Steveninck *et al*, 1993). EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 KOHM. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Data blocks containing artefacts were identified and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the very low (0.5–2 Hz), δ (2–4 Hz), ϑ (4–7.5 Hz), α (7.5–13.5 Hz), β (13.5–35 Hz), and γ (35–48.9 Hz) frequency ranges. The duration of EEG measurements was 64 seconds per session.

PUPIL SIZE ✳ Pupil diameter was determined using a digital camera (Canon powershot A620) and a flash. The subject was instructed to look into the lens. A sharp picture of the eyes was taken using a camera with flash. All pictures were stored digitally. The diameters of the pupil and the iris were determined in the number of pixels used horizontally. For each eye, these values were recorded on data collection forms, and the pupil / iris ratio was subsequently calculated as a measure of pupil size.

BODY SWAY ✳ The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with a pot string meter (celesco) based on the Wright ataxia meter (Wright, 1971). This method has been used to demonstrate effects of sleep deprivation (van Steveninck *et al*, 1999), alcohol (van Steveninck *et al*, 1993) and benzodiazepines (van Steveninck *et al*, 1993; Van Steveninck *et al*, 1997). With a string attached to the waist, all body movements over a period of time were integrated and expressed as mm sway. The total period of body-sway measurement was two minutes.

STROOP ✳ The Stroop test mainly investigates inhibition, interference and controlled versus automatic processing. A two trial version of the colour-word Stroop task was presented to the subjects. In the first trial, six coloured items in green, red or blue were presented at random and subjects indicated which colour they saw. In the second trial, 34 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were asked to indicate the colour of the word (for example: if the word blue was written in red, the correct answer was 'red') (Laeng *et al*, 2005).

SIMPLE REACTION TIME TASK ✳ The Simple Reaction Time Task (SRTT) measures the attention and speed of information processing of the participant. In this task, participants view a black computer screen. At random intervals (0.5–1.5 seconds), a white circle appears in the centre of the computer screen. Participants were instructed to press the space bar with the index finger of their dominant hand each time the circle appears. They were instructed to respond as quickly as possible after appearance of the circle. A total of 40 circles were presented, and the duration of the task was approximately 1 minute. The outcome of the task is the time between stimulus display and response. It has been shown to respond to several classes of sedative drugs (Wezenberg *et al*, 2007).

VISUAL ANALOGUE SCALE ✳ Changes in subjective conditions are important aspects of drug effects, and a visual analogue scale (VAS) is one of the most commonly used ways to assess subjective states. It is a psychometric response scale, which is particularly suited to repeatedly quantify present subjective states. In the VAS according to Bond & Lader, the 'directions' of different scales on a form were alternated, to avoid 'habitual scoring' by subjects. Composite scores were derived for alertness, mood and calmness (Norris, 1971).

VISUAL VERBAL LEARNING TEST ✳ The Visual Verbal Learning Test (VVLT) contains three different subtests that cover almost the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval) (de Haas *et al*, 2009). Subjects were presented 30 words in three consecutive word trials. Each trial ended with a free recall of the presented words (Immediate Recall). Approximately thirty minutes after start of the first trial, the volunteers were asked to recall as many words as possible (Delayed Recall). Immediately thereafter, the volunteers underwent memory recognition test, which consisted of 15 presented words and 15 'distractors' (Recognition).

SAFETY ASSESSMENTS

All subjects underwent medical screening, including medical history, physical examination, vital signs measurement in supine and standing position, 12-lead electrocardiogram (ECG), urinalysis, drug screen and safety chemistry and haematology blood sampling. During study periods, safety was assessed using monitoring of adverse events, vital signs, ECG and safety chemistry and haematology blood sampling.

PHARMACOKINETIC AND STATISTICAL ANALYSIS

The graphs and the pharmacokinetic parameters for mecamylamine were calculated by non-compartmental analysis in R (R Core Team, 2013). Primary pharmacokinetic endpoints were: maximum plasma concentration (C_{max}), time of maximum plasma concentration (T_{max}), area under the plasma concentration vs. time curve ($AUC_{0\text{-}last}$), area under the plasma concentration vs. time curve extrapolated to infinity ($AUC_{0\text{-}\infty}$), apparent terminal half-life, apparent clearance (CL/F) and apparent volume of distribution (Vd/F).

A mixed model analysis of covariance using SAS 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA) was used for analyses of pharmacodynamic effects, with subject, subject by treatment and subject by time as random effects; treatment, study period and by treatment by time as fixed effects; and the average baseline value as covariate. VVLT was analysed using a mixed model analysis of variance with fixed factors treatment and period, random factor subject and, if available, the (average) baseline. As this was an exploratory study, no formal adjustment for multiple testing was used. A p value below 0.05 was considered statistically significant. In order to properly compare scopolamine and mecamylamine effects, two timepoints before scopolamine administration (1 and 2 hours after mecamylamine administration) were not included in the LSM graphs.

RESULTS

A total of 15 healthy male subjects participated in the trial. During execution of the study, three subjects stopped prematurely, due to personal circumstances

(1), difficulties in blood sampling (1) and because of adverse events (nausea; 1). A total of 14 subjects completed at least one study period with treatment of mecamylamine and 12 subjects completed all study occasions. Subjects had a mean age of 25.9 (range 19–36) years, weight of 80.9 (range 59.9–90.0) kg and BMI of 24.4 (range 18.6–30.3) $kg \cdot m^{-2}$.

SAFETY

All subjects reported at least one treatment emergent adverse event. Most frequent occurring adverse events were somnolence, dizziness, fatigue, nausea, dry mouth and headache (table 3.1). Adverse effects were mild and occasionally moderate and all disappeared spontaneously within a few hours. 3 of 14 subjects reported postural dizziness at the 20 mg mecamylamine dose. This coincided in all cases with measurable orthostatic hypotension.

The difference between standing and supine blood pressure significantly increased on the 20 mg mecamylamine dose, compared to placebo, while heart rate was significantly higher (table 3.2). Also, the difference in blood pressure between supine and standing position was significantly higher on the 20 mg mecamylamine dose, compared to placebo. On the 10 mg dose of mecamylamine, only the increase in supine and standing heart rate was statistically significant compared to placebo. There were no other consistent changes in ECG or laboratory safety parameters.

PHARMACOKINETICS

The mean T_{max} of mecamylamine was 2.1 hours (range 1–3.3) with a C_{max} of 33.9 $ng \cdot ml^{-1}$ (range 23.4–44.1) for the 10 mg dose and 2.5 hours (range 0.5–6) with a C_{max} of 64.5 $ng \cdot ml^{-1}$ (range 45.9–80.1) for the 20 mg dose (table 3.3). When analysing the individual plots. The terminal half-life was estimated to be 8.5 hours for 10 mg and 11.7 hours for 20 mg mecamylamine. This difference was not statistically significant. Other pharmacokinetic parameters were estimated as follows: CL/F = 17.9 $L \cdot h^{-1}$ (range 15.1–20.7) and Vd/F = 283 L (range 260–307).

Scopolamine pharmacokinetics could not be described in detail due to the low sample frequency after administration of scopolamine. The mean C_{max} of scopolamine was 2549 $pg \cdot ml^{-1}$ (range 1349–4835) measured 15 minutes after the start of scopolamine infusion in all subjects. This is consistent with a previously published PK model of scopolamine (Liem-Moolenaar *et al*, 2011).

PHARMACODYNAMICS

The main outcome parameters of the pharmacodynamic effects are summarized in table 3.4 and figure 3.1; more detailed information is reported in the supplementary material. Both administration of scopolamine and the 20 mg dose of mecamylamine led to a significant decrease compared to placebo in performance on adaptive tracking, the second and third trial of the immediate recall and the delayed recall of the visual verbal learning test (figure 3.2), finger tapping, body sway and VAS alertness. The effects of scopolamine were significantly stronger than those of mecamylamine on all these parameters, except for finger tapping and body sway. In contrast to mecamylamine, scopolamine administration resulted in an increase in reaction time and an increased score on the VAS for calmness compared to placebo. Scopolamine also induced a decrease in performance on all N-back parameters, a decrease in alpha and beta power on the p-EEG, and a decreased performance on the first immediate recall and the delayed recognition of the VVLT, the SRT and saccadic peak velocity and accuracy and smooth pursuit eye movements, while mecamylamine administration did not affect these tests. On the Stroop test, mecamylamine administration led to a decrease in reaction time compared to placebo, while scopolamine led to an increase in performance. Saccadic reaction time only increased after administration mecamylamine. No consistent differences between mecamylamine and placebo could be observed for N-back, SRT, p-EEG, saccadic inaccuracy, saccadic peak velocity, smooth pursuit eye movements and VAS Calmness. Reaction time on the VVLT recognition, pupil size and VAS mood were not affected by either scopolamine or mecamylamine compared to placebo.

DISCUSSION

In this study, we investigated the pharmacodynamic and pharmacokinetic profile over time of mecamylamine using an extensive CNS test battery that included cognitive as well as visuomotor and neurophysiological measures. Two oral doses of mecamylamine were compared to intravenously administered scopolamine and placebo in order to determine the profile of a nAChR specific anti-cholinergic pharmacological challenge model. All treatments administered were considered safe and well tolerated, since all adverse events were transient and mild to moderate in severity. Pharmacokinetics of scopolamine are in line with previously described results (Liem-Moolenaar *et al*, 2011). The plasma concentrations of mecamylamine almost doubled with the doubling of the dose, which suggests dose-proportionality, as has been described before (Young *et al*, 2001).

Mecamylamine showed a dose dependent decrease in performance on several tests that represent different cognitive domains. The decline in performance on adaptive tracking and reduced VAS alertness reflected a deficiency in sustained attention. The decrease on the third trial of the immediate and the delayed recall of the VVLT represents a reduction in learning ability and memory retrieval. This mecamylamine induced impairment in acquisition and recall of information was expected, based on the localisation of nAChRs the brain (Paterson and Nordberg, 2000). These effects last up to 10 hours after drug administration. Mecamylamine did not have any significant effects on measures for sedation (SRTT and saccadic peak velocity).

The cognitive effects of mecamylamine found in this study are consistent with previous research, where mecamylamine was administered at doses of 5, 10 and 20 mg to healthy young and elderly volunteers (Newhouse *et al*, 1992, 1994). In these studies, the effects on cognition were studied one and two hours after dosing. A dose-dependent decrease in learning ability and reaction time was reported, which was more pronounced in elderly volunteers. There was no effect on subjective scales for drowsiness. Another study reported significant decrease in learning ability and semantic memory after administration of 15 mg mecamylamine (Little *et al*, 1998) and also a decrease in inspection time after administration of 20 mg of mecamylamine was reported (Thompson *et al*, 2000). Cognitive testing was done at one (Little *et al*, 1998; Thompson *et al*, 2000) or two (Newhouse *et al*, 1992, 1994) time points after dosing and tests for sustained attention were not performed in these studies. In none of the previously mentioned studies plasma mecamylamine concentrations were measured.

Conversely, several other studies found no effects of mecamylamine on various cognitive tests (Dumas *et al*, 2008; Ellis *et al*, 2006; Erskine *et al*, 2004; Green *et al*, 2005; Thienel *et al*, 2009; Voss *et al*, 2010). However, these studies all used a dose of 15 mg and investigated the cognitive effects at only one time point after dosing. With this relatively low dose and measurements at only one time point, modest effects may have been missed. This is supported by the finding that the attentional network measured with fMRI was down regulated after administration of the same dose of mecamylamine, while cognitive tests were not influenced (Dumas *et al*, 2010; Thienel *et al*, 2009). The slightly higher dose of mecamylamine and the frequency and sensitivity of our test may have attributed to the positive results of our study.

The second aim of this study was to compare the mecamylamine model with the anti-muscarinic scopolamine model. Several previous studies attempted to do this before, but none of these studies found significant cognitive effects of mecamylamine to compare with, probably due to low doses and few measurements (Dumas *et al*, 2008; Ellis *et al*, 2006; Erskine *et al*, 2004; Green *et al*, 2005; Little *et al*, 1998; Voss *et al*, 2010). In this study, scopolamine had a significant effect on all cognitive domains measured, including inhibition and working memory, as has been described before (Broks *et al*, 1988; Ellis *et al*, 2006; Green *et al*, 2005; Liem-Moolenaar *et al*, 2011; Little *et al*, 1998). The increase in reaction time and decrease in saccadic peak velocity, which was not observed after mecamylamine administration, and the larger reduction of VAS alertness, suggest that scopolamine has a strong sedative effect. These sedative effects of scopolamine have been previously reported (Kamboj and Curran, 2006; Koller *et al*, 2003; Pergolizzi *et al*, 2012). It is unlikely that this is related to relative dose differences between the doses of mecamylamine and scopolamine given in this study, since sedation is also reported after lower doses of scopolamine (Koller *et al*, 2003) and mecamylamine has been given as antihypertensive in doses up

to 80 mg in the past without any relevant sedation. The brainstem and basal brain areas controlling arousal and wakefulness contain more mAChR than nAChR (Brown *et al*, 2012), which is a likely explanation for the difference in sedative effects between mecamylamine and scopolamine. The scopolamine induced sedation may contribute to the cognitive effects of scopolamine in this study which are more pronounced than those of mecamylamine (Ford *et al*, 1956; MCQUEEN and SMIRK, 1957). The larger magnitude of the effects of scopolamine may seem attractive, but smaller, though still relevant effects of a new compound might get lost in the margins of variability or get overshadowed by the sedation caused by scopolamine. Due to the absence of sedation, the mecamylamine challenge may not only be more suitable for proof of pharmacology studies with a nAChR agonist, but also for other procognitive compounds.

We can conclude from this study that the nicotinic anticholinergic pharmacological challenge with mecamylamine results in measurable cognitive deficits with a nAChR specific profile, which is clearly distinguishable from the profile of the mAChR antagonist scopolamine. The mecamylamine challenge could therefore be suitable for proof of pharmacology studies with nAChR agonists. Furthermore, the relevant lack of sedation is an advantage of the mecamylamine challenge, compared with the scopolamine challenge.

A PK-PD-model of mecamylamine would be helpful in designing studies with the mecamylamine challenge. However, with the results of this study, PK-PD-modelling of the neurophysiological endpoints was not possible due to the narrow range of difference in pharmacodynamic effects between the mecamylamine lower and higher dose.

In conclusion, this study demonstrated that mecamylamine causes nicotinic receptor specific temporary decline in cognitive functioning and affects different CNS domains. Compared with the scopolamine model, pharmacodynamic effects were less pronounced at the dose levels tested and caused less sedation. Whether the mecamylamine model can be used for proof-of-pharmacology of nicotinic acetylcholine receptor agonists remains to be established.

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TABLE 3.1 Most frequent treatment emergent adverse events.

Number of adverse events and percentage from the subjects experiencing the adverse events.

	Placebo n=14	Mecamylamine 10 mg n=12	Mecamylamine 20 mg n=14	Scopolamine 0.5 mg n=13
Subjects with at least 1 AE	7 (50.0%)	8 (66.7%)	12 (85.7%)	13 (100%)
Number of different AEs	8	9	33	19
Somnolence	2 (14.3%)	6 (50.0%)	9 (64.3%)	7 (53.8%)
Dizziness	-	2 (16.7%)	4 (28.6%)	10 (76.9%)
Fatigue	2 (14.3%)	2 (16.7%)	5 (35.7%)	4 (30.8%)
Nausea	2 (14.3%)	1 (8.3%)	5 (35.7%)	3 (23.1%)
Dry mouth	1 (7.1%)	-	1 (7.1%)	5 (38.5 %)
Headache	2 (14.3%)	2 (16.7%)	1 (7.1%)	2 (15.4%)
Disturbance in attention	-	1 (8.3%)	2 (14.3%)	1 (7.7%)
Dysgeusia	1 (7.1%)	-	2 (14.3%)	1 (7.7%)
Diplopia	-	-	1 (7.1%)	2 (15.4%)
Dizziness postural	-	-	3 (21.4%)	-

TABLE 3.2 Vital signs per treatment group.

Per group the difference estimate and in parenthesis the confidence interval is presented.

	Treatment effect	Mecamylamine 10 mg n=12	Mecamylamine 20 mg n=14	Scopolamine 0.5 mg n=13
Diastolic BP (supine) (mmHg)	p = 0.1372	1.5 (-1.2, 4.2) p=0.2674	-0.6 (-3.1, 2.0) p=0.6652	-1.7 (-4.3, 1.0) p=0.2067
Diastolic BP (standing) (mmHg)	p = 0.0021	0.1 (-3.4, 3.5) p=0.9682	-6.2 (-9.5,-2.8) p=0.0007	-2.2 (-5.7, 1.2) p=0.1995
Diastolic BP (standing-supine) (mmHg)	p = 0.0028	-1.0 (-4.3, 2.3) p=0.5428	-5.5 (-8.6,-2.5) p=0.0009	-0.3 (-3.4, 2.9) p=0.8698
Systolic BP (supine) (mmHg)	p = 0.0379	-0.4 (-4.0, 3.3) p=0.8436	-4.5 (-8.0,-0.9) p=0.0149	-3.4 (-7.0, 0.2) p=0.0632
Systolic BP (standing) (mmHg)	p = 0.0030	-1.7 (-6.0, 2.6) p=0.4277	-7.8 (-12.0,-3.7) p=0.0005	-1.6 (-5.9, 2.7) p=0.4507
Systolic BP (standing-supine) (mmHg)	p = 0.0129	-1.7 (-5.3, 1.9) p=0.3445	-4.9 (-8.4,-1.3) p=0.0090	0.8 (-2.8, 4.5) p=0.6441
Heart rate (supine)(bpm)	p < 0.0001	6.9 (3.4,10.3) p=0.0003	9.4 (6.3,12.6) p<0.0001	-4.5 (-7.8,-1.2) p=0.0099
Heart rate (standing) (bpm)	p < 0.0001	8.7 (2.9,14.5) p=0.0042	16.0 (10.4,21.5) p<0.0001	-4.4 (-10.3, 1.5) p=0.1390

TABLE 3.3 Summary of mecamylamine pharmacokinetic parameters.

	Mecamylamine 10 mg (n=12)				Mecamylamine 20 mg (n=14)			
Characteristic	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
C_{max} (ng/ml)	33.9	5.96	23.4	44.1	64.5	10.9	45.9	80.1
T_{max} (hr)	2.05	0.92	1	3.28	2.57	1.61	0.5	6
Terminal half life (hr)	8.48	1.47	5.44	11.22	11.66	5.41	6.16	23.9
$AUC_{0\text{-inf}}$	503.8	126.3	332.9	746.1	1346.1	564.7	672.3	2621.8
$AUC_{0\text{-last}}$	410.1	90.0	277.7	607.0	913.8	187.3	603.5	1260.6

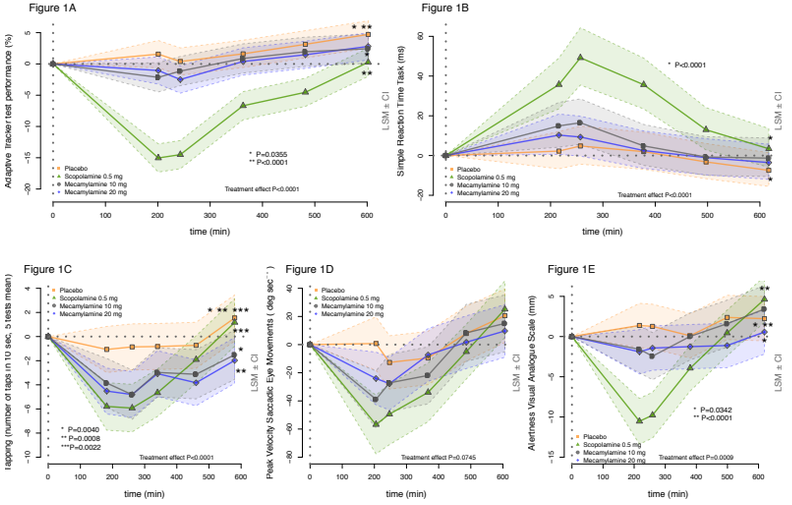
TABLE 3.4 Pharmacodynamic effects on cognitive tests.

Per group the difference estimate and in parenthesis the confidence interval is presented.

	Treatment effect	Mecamylamine 10 mg n=12	Mecamylamine 20 mg n=14	Scopolamine 0.5 mg n=13
Adaptive tracking (%)	p < 0.0001	-1.89 (-3.90, 0.12) p=0.0647	-2.06 (-3.97,-0.15) p=0.0355	-10.4 (-12.4,-8.39) p<0.0001
VAS alertness (mm)	p = 0.0009	-1.3 (-3.7, 1.2) p=0.2962	-2.5 (-4.8,-0.2) p=0.0342	-5.3 (-7.7, -2.9) p<0.0001
Finger tapping (taps in 10 sec)	p = 0.0025	-2.87 (-4.75,-0.99) p=0.0040	-3.25 (-5.05,-1.46) p=0.0008	-3.04 (-4.89,-1.18) p=0.0022
VVLT 3rd recall (number of words)	p < 0.0001	-2.7 (-5.1, -0.3) p=0.0286	-3.6 (-5.9,-1.4) p=0.0025	-7.7 (-10.1, -5.4) p<0.0001
VVLT delayed recall (number of words)	p < 0.0001	-3.1 (-5.8, -0.4) p=0.0259	-3.8 (-6.4,-1.2) p=0.0051	-7.1 (-9.8, -4.5) p<0.0001
Simple reaction time task (% change)	p < 0.0001	7.0% (-0.8%, 15.5%) p=0.0786	3.8% (-3.5%, 11.7%) p=0.3080	26.8% (17.6%, 36.8%) p<0.0001
Saccadic peak velocity (deg·sec-1)	p = 0.0745	-14.3 (-33.5, 4.8) p=0.1367	-10.9 (-29.0, 7.1) p=0.2232	-25.4 (-44.2, -6.6) p=0.0098

FIGURE 3.1 Effect on Tests Evaluating Fine Coordination, Reaction Time, Alertness, Motor Fluency and Eye Movements.

Mecamylamine 10 mg, mecamylamine 20 mg, scopolamine 0.5 mg or placebo effect versus time during the Adaptive Tracking test, Simple Reaction Time Task, Tapping, Peak Velocity of the Saccadic Eye Movements and the Visual Analogue Scale evaluating Alertness.



Symbols represent the least square means per treatment group and the polygon (shaded area around the mean) the predicted confidence interval. Asterisks represent significance between groups (p value is mentioned per overall treatment effect and per group, when applicable). Vertical discontinuous line represents time point zero and the horizontal line represents zero.

FIGURE 3.2 Effect on Tests Evaluating Retrieval.

Mecamylamine 10 mg, mecamylamine 20 mg, scopolamine 0.5 mg or placebo effect versus time during the Delayed Word Recognition and the number of correct answers during the third Recall condition of the Verbal Visual Learning Test. The box plots represent the first and third quartile, the middle line the group mean and the 'M' represents the median. The vertical lines the confidence interval. Individual observations are plotted as well.