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Cardiac safety profile of etamicastat, a novel peripheral selective dopamine-β-hydroxylase inhibitor in non-human primates, human young and elderly healthy volunteers and hypertensive patients

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ABSTRACT

The aim of this work was to evaluate the cardiac risk for etamicastat, a peripheral reversible dopamine-β-hydroxylase inhibitor. Etamicastat blocked the hERG current amplitude with an IC_{50} value of 44 μg/ml. Etamicastat had no substantial effects on arterial blood pressure, heart rate and the PR interval in male *Cynomolgus* monkeys when administered orally up to 90 mg/kg. Administered orally at 15 and 45 mg/kg/day in female and male *Cynomolgus* monkey for 91 days, etamicastat had no effect on heart rate and the waveform or intervals of the electrocardiogram. At the highest dose level of 45 mg/kg, mean plasma concentrations of etamicastat ranged from 1875 to 3145 ng/ml on Day 1 and Day 91 of treatment, respectively. The effect of age on the tolerability and pharmacokinetics of etamicastat in elderly (≥65 years) and young adult (18–45 years) subjects showed that supine systolic (SBP) and diastolic (DBP) blood pressure, ECG heart rate, PR interval, QRS duration and QTcF interval were not affected following once-daily administration of 100 mg/day etamicastat for 7 days. In hypertensive patients the decrease of blood pressure tended to be more important in subjects who had received etamicastat (50, 100 and 200 mg) than in subjects who had received placebo. No clinically significant out-of-range values in vital signs or ECG parameters, ECG heart rate, PR interval, QRS duration and QTcF interval were observed in hypertensive subjects following once-daily administration of etamicastat for 10 days. In conclusion, etamicastat is not likely to prolong the QT interval at therapeutic doses.

1. Introduction

Interest in the development of inhibitors of dopamine β-hydroxylase (DβH; EC 1.14.17.1; dopamine β-monooxygenase) is centered on the hypothesis that inhibition of this enzyme may provide significant clinical improvements in patients suffering from cardiovascular disorders such as hypertension or congestive heart failure [1–8]. The rationale for the use of DβH inhibitors is based on their capacity to inhibit the biosynthesis of noradrenaline, which is achieved via enzymatic hydroxylation of dopamine [9–13].

Several DβH inhibitors have been reported [4–6], but none achieved marketing approval due to weak potency, poor DBH selectivity [14] and/or significant adverse effects [15]. Nepicastat, (Fig. 1) a 5-substituted imidazole-2-thione derivative, is a highly potent DβH inhibitor that, in beagle dogs, produced a dose-dependent noradrenaline reduction and dopamine increase in the renal artery, heart left ventricle and cerebral cortex [7]. These data indicate that nepicastat crosses the blood–brain barrier (BBB) causing central as well as peripheral effects, a situation that could lead to undesired and potentially serious CNS adverse effects. Etamicastat, also known as BIA 5–453, (Fig. 1) is a reversible DβH inhibitor that prevents the conversion of dopamine to noradrenaline in sympathetically innervated tissues and reduces sympathetic nervous system activity [1,2,14]. As a result of its reduced ability to cross the blood–brain barrier [1], etamicastat acts preferentially in the periphery and is currently being developed for the treatment of cardiovascular diseases.

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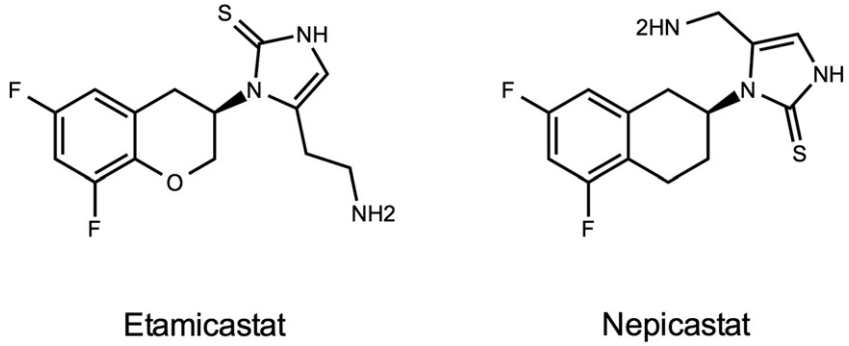

Etamicastat Nepicastat

Fig. 1. Structural formulae of etamicastat and nepicastat.

In contrast to the effects in peripheral tissues, etamicastat failed to affect dopamine and noradrenaline tissue levels in the brain [1], which is unique among the DβH inhibitors previously tested for the treatment of cardiovascular disorders [3,8,14].

As previously observed with other DβH inhibitors that are endowed with potent antihypertensive effects in the spontaneously hypertensive rat [6], etamicastat showed to reduce both systolic (SBP) and diastolic (DBP) blood pressure, alone or in combination with other antihypertensive drugs, and to decrease the urinary excretion of noradrenaline in spontaneously hypertensive rats with no change in heart rate [16–19]. Recently, etamicastat demonstrated blood pressure lowering effects in hypertensive patients [20]. In healthy subjects, etamicastat was well tolerated and showed approximate linear pharmacokinetics following single oral doses [21] and multiple once-daily oral doses [22], with no significant differences being observed in elderly versus young healthy subjects [23].

The aim of the present work was to evaluate the effects of etamicastat for cardiac risk both in vitro, testing on the hERG potassium channel of human embryonic kidney (HEK293) cells, and in vivo in the *Cynomolgus* monkey monitored by telemetry (up to 90 mg/kg etamicastat). This evaluation was complemented in male and female *Cynomolgus* monkey dosed daily with etamicastat (up to 45 mg/kg/day) for at least 91 days, and in three clinical studies in human healthy volunteers receiving single doses of etamicastat (up to 1200 mg), young and elderly healthy subjects receiving etamicastat (100 mg/day) for 7 days, and hypertensive patients administered once-daily with etamicastat (up to 200 mg) for up to 10 days.

2. Methods

2.1. In vitro studies

2.1.1. DβH activity

DβH activity was measured by a modification of the method of Nagatsu and Udenfriend [24], which is based on the enzymatic hydroxylation of tyramine into octopamine, in SK-N-SH cell homogenates. SK-N-SH cells (ATCC HTB-11) obtained from LGC Standards (Tedington, UK) were cultured in Eagle's minimum essential medium supplemented with 25 mM Hepes, 100 U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin and 10% Gibco® fetal bovine serum. Cells were grown in T162 cm flasks (Corning, NY) in a humidified atmosphere of 5% CO_2–95% air at 37 °C. For the preparation of homogenates, fetal bovine serum was removed from cell medium 4 h prior to homogenate preparation. At the appropriate time media was removed and cell monolayers were washed with 50 mM Tris–HCl pH 7.4. Cells were subsequently scrapped off the flasks and were resuspended in 50 mM Tris pH 7.4. Cell suspensions were homogenized with SilentCrusher M (Heidolph) for a short stroke and homogenates were aliquoted and were stored frozen at −80 °C. Total protein in cell homogenates was determined with the BioRad Protein Assay (BioRad) using a standard curve of BSA (50–250 μg/ml). The octopamine formed is subsequently oxidized to *p*-hydroxybenzaldehyde and measured by spectrophotometry. Experimental assay conditions for the cellular homogenates were previously optimized by evaluating time and protein dependency of the enzymatic assay. In brief, reaction mixture (total volume 500 μl) contained: cellular homogenate (75 μg total protein) sodium acetate pH 5.0 (200 mM), NEM (30 mM), $CuSO_4$ (5 μM), catalase aqueous solution (0.5 mg/ml), pargyline–HCl (1 mM), sodium fumarate (10 mM), ascorbic acid (10 mM), inhibitor or vehicle and tyramine (25 mM). After a 10 min pre-incubation period at 37 °C, the reaction was initiated by the addition of tyramine. Reaction was carried out for 45 min at 37 °C before termination with 50 μl PCA (2 M). Samples were centrifuged for 3 min at 16,100 *g* and supernatants were transferred to SPE cartridges ISOLUTE SCX-3 (100 mg, 1 ml) previously equilibrated with MilliQ water. Columns were centrifuged at 150 *g* for 2 min. Eluate was discarded and matrix was washed with 1 ml of MilliQ water after which octopamine was eluted with 2 × 0.25 ml ammonium hydroxide (4 M). The oxidation of octopamine to *p*-hydroxybenzaldehyde was carried out for 6 min with 100 μl sodium periodate (2%) and was stopped with 100 μl sodium metabisulfite (10%). Absorbance was measured at 330 nm on a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA). Under the experimental conditions described in above, cellular homogenates were incubated with various concentrations (1, 3, 10, 30, 100, 300, 1000, 3000 nM) of either etamicastat, or nepicastat.

2.1.2. hERG K^+ channel

The whole-cell patch-clamp technique was used to investigate the effects of etamicastat and nepicastat on hERG potassium channels stably expressed in stably transfected human embryonic kidney (HEK 293) cells. Both compounds were tested at concentrations of 0.3 to 35.0 μg/ml in order to determine their effects on the hERG mediated current. All solutions applied to cells including the pipette solution were maintained at room temperature (19–30 °C). A vehicle group (water for injection 1% used as solvent for etamicastat and nepicastat) was included in the study for comparison, and E-4031 (1 μM), which selectively blocks the rapid delayed rectifier potassium current IKr, was used as reference substance.

HEK 293 cells stably expressing the hERG channel were incubated at 37 °C in a humidified atmosphere with 5% CO_2. For electrophysiological measurements, HEK 293 cells were seeded onto 35 mm sterile culture dishes containing 2 ml culture medium without antibiotics. Tetracycline was added to induce channel expression. Because responses in distant cells are not adequately voltage clamped and because of uncertainties about the extent of coupling [25], cells were cultivated at a density that enabled single cells (without visible connections to neighboring cells) to be measured. The cells were continuously maintained in and passaged in sterile culture flasks containing a 1:1 mixture of Dulbecco's modified eagle medium and nutrient mixture F-12 (D-MEM/F-12 1×, liquid, with GlutaMax I, Gibco-BRL) supplemented with 9% fetal bovine serum (Gibco-BRL) and 0.9% penicillin/streptomycin solution (Gibco-BRL). The complete medium as indicated above was supplemented

with 100 mg/ml hygromycin B (Invitrogen) and 15 mg/ml Blasticidin (Invitrogen). The final bath solution had the following composition (in mM): NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, D-Glucose 10, pH (NaOH) 7.4. The pipette solution had the following composition (in mM): KCl 130, $MgCl_2$ 1, Mg-ATP 5, HEPES 10, EGTA 5, pH (KOH) 7.2. The 35 mm culture dishes upon which cells were seeded at a density allowing single cells to be recorded were placed on the dish holder of the microscope and continuously perfused (approximately 1 ml/min) with the bath solution. All solutions applied to cells including the pipette solution were maintained at room temperature (19–30 °C). After formation of a Gigaohm seal between the patch electrodes and individual hERG stably transfected HEK 293 cells (pipette resistance range: 2.0 MW–7.0 MW; seal resistance range: >1 GW), the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior (whole-cell patch-configuration). As soon as a stable seal was established, hERG outward tail currents were measured upon depolarization of the cell membrane to + 20 mV for 2 s (activation of channels) from a holding potential of − 80 mV and upon subsequent repolarization to − 40 mV for 3 s. This voltage protocol was run at least 10 times at intervals of 10 s. If current density was judged to be too low for measurement, another cell was recorded. Once control recordings had been accomplished, cells were continuously perfused with a bath solution containing etamicastat at 3, 10, 30 and 100 μM. During wash-in of the test item, the voltage protocol indicated above was run continuously again at 10 s intervals until the steady-state level of block was reached. Complete cumulative concentration-response analysis was accomplished per cell and the IC_{50} value was calculated. After measurement of the control period, concentrations of etamicastat were applied to the perfusion bath. During wash-in of etamicastat, the voltage protocol was run until the steady-state level of channel inhibition was reached. Values (in pA/nA) of the peak amplitudes of outward tail currents were generated for each voltage step. The recorded current amplitudes at the steady-state level of current inhibition were compared to those from control conditions measured in the pre-treatment phase of the same cell. The amount of current block was calculated as percentage of control. To determine whether any observed current inhibition was due to etamicastat interaction with the hERG channel or due to current rundown, these residual currents were compared to those measured in vehicle treated cells. Data from 3 individual cells were collected and the corresponding mean values and standard errors calculated. For the validation of the test system, the selective I_{Kr} blocker E-4031 was evaluated at 100 nM in 3 cells.

2.2. In vivo studies

2.2.1. Cynomolgus primates

For cardiac and pharmacokinetic assessments, male and female *Cynomolgus* monkeys, with bodyweight ranging from 1.8 to 2.7 kg (females) and 2.2–3.1 kg (males), were obtained from Bioculture Mauritius Ltd. (Senneville, Riviere des Anguilles, Mauritius) or Guangxi Grand forest Scientific Primate Company (Beijing, China). The animals were housed in group cages. Each cage contained all animals of the same sex and treatment group. The animal house was maintained under a 12-h fluorescent light/12-h dark cycle at a controlled ambient temperature of 20–24 °C and relative humidity ranged from 40 to 70%. Animal diet consisted of pelleted standard Teklad diet 2055 and 2055C monkey diet (150 g/day/animal) and a fresh piece of fruit four times a week. During the treatment period, the diet was offered approximately 1 h after completion of dosing. Any remaining diet was withdrawn early on the following day. All animal interventions were performed in accordance with the European Directive number 86/609, and the rules of the "Guide for the Care and Use of Laboratory Animals", 7th edition, 1996, Institute for Laboratory Animal Research (ILAR), Washington, DC.

2.2.1.1. Telemetry monitored evaluations in Cynomolgus primates. The effects of etamicastat (15, 45 and 90 mg/kg) on arterial blood pressure, heart rate and the main parameters of the electrocardiogram were evaluated following oral (p.o. capsule) administration in the conscious male *Cynomolgus* primate monitored by telemetry. The doses of etamicastat were based on the results from a maximum tolerated dose (MTD) study in *Cynomolgus* monkeys in which the MTD was established as 120 mg/kg/day (BIAL data on file). There was 1 treatment group of 4 primates following a 4 × 4 Latin-square design. There was a washout period of 1 week between each treatment. Animals were dosed at approximately the same time each day. Venous blood samples (approximately 1 ml) for determination of etamicastat in plasma were taken prior to dosing and at 2 and 4 h after the start of dose administration, or as close as was reasonably practicable to these time points.

Four male *Cynomolgus* primates were surgically implanted with telemetry transducers, type TL11M2-D70-PCT (Data Sciences International), for the measurement of arterial blood pressure and lead II electrocardiogram (ECG). Briefly, using sterile techniques and with the animals anaesthetized with isofluorane, the implant body was placed intraperitoneally in the lower quarters of the abdominal cavity and the blood pressure catheter introduced into the femoral artery with the tip estimated to be in the terminal abdominal aorta. The ECG electrodes were tunneled subcutaneously and fixed in the thorax to give a lead II ECG waveform morphology. Approximately 3 weeks later, i.e. after a recovery period during which visual inspection of waveform morphology of arterial blood pressure and of the ECG was performed at times to confirm suitability of the animals, a telemetry receiver was positioned nearby each animal's home cage to record mean blood pressure (MBP), SBP and DBP and heart rate (HR), which was derived from pulse blood pressure. The PR and the QT intervals (ms) were also measured and the QTc interval was calculated according to Fridericia's formula [$QTcF = QT\ (ms) / \sqrt[3]{[60/HR(bpm)]}$], and to individual animal specific correction formula [$QTcQ = QT\ (ms) + \#(500\text{-}RR)$]. Arterial blood pressure, heart rate and lead II ECG variables in all groups were extracted at − 0.5, 1, 2, 4, 6, 8, 10, 14, 18 and 22 h post-dose, where time 0 is the time at the end of dosing. Etamicastat was examined at 3 ascending single doses, i.e. 15, 45 and 90 mg/kg, administered p.o. (as powder using gelatine capsules). Each animal received the vehicle (empty capsule), then etamicastat, with a washout period of at least 5 days between each treatment. The animals were offered food between approximately 2 h post-dosing, on completion of the blood sampling. Approximately 1 ml of venous blood was collected from a jugular vein into lithium-heparinized glass vacuum tubes before and 2 and 4 h after the administration of etamicastat or vehicle; the tubes were stored in ice until centrifugation (approximately 1500 g at 4 °C for 15 min) and until required for analysis.

2.2.1.2. Cardiac evaluation after repeated oral administration in Cynomolgus primates. Four animals of each sex were dosed daily by oral capsule at 0, 15 and 45 mg/kg/day for at least 91 days. On each day of treatment, the animals were housed individually immediately prior to and after dosing to allow detection of any treatment-related clinical signs. Thereafter, the animals were released into group cages to allow interaction within the dose group and sex. Animals were dosed orally by gastric gavage and individual doses were adjusted weekly according to the most recently recorded bodyweight. ECGs were recorded for all animals before the start of treatment and again before dosing and 1 h after dosing on one day during Week 1 and Week 13 of the study. Electrocardiograms were obtained using Einthoven (I, II and III) and Goldberger (aVR, aVL, aVF) leads. The heart rate, P wave duration and amplitude, P–Q interval, QRS interval and Q–T intervals were measured manually using a representative section of the electrocardiogram from lead II, on the basis of an average of at least 10 consecutive complexes data.

Blood samples for the determination of plasma concentrations of etamicastat were taken, by direct vein puncture, into lithium-heparin tubes at pre-dose and then at 1, 2, 4, 8, 12 and 24 h after dosing. After

collection, blood samples were centrifuged at approximately 1500 *g* for 10 min at 4 °C. The resulting plasma was then separated into 2 aliquots of 250 μl and stored at −80 °C until required for analysis.

2.2.2. Human subjects

The healthy status of human volunteers was determined by an interview, medical history, physical examination, vital signs, 12-lead ECG, and results of clinical laboratory safety tests (including hematology, plasma biochemistry), urinalysis, drugs of abuse screen, and HIV and hepatitis B and C serology considered clinically acceptable at screening and admission. At admission to the unit, the medical history and physical examination were updated. Etamicastat was administered in the morning, with 250 ml of water, after at least 8 h of fasting. The doses were obtained as combinations of etamicastat 1 mg, 10 mg and 50 mg capsules and placebo capsules identical in appearance manufactured by BIAL (S. Mamede do Coronado, Portugal). Healthy subjects participating in the single ascending dose study were requested to abstain from consuming grapefruit or other citrus (e.g. orange) or their juice, and alcohol- or xanthine-containing beverages or foods until 120 h after the last dose. Hypertensive patients participating in the hypertension study were required to abstain from strenuous physical activity, smoking, consumption of grapefruit or other citrus fruit (e.g. orange) or of their juice, alcohol and beverages containing xanthine derivatives (i.e. no coffee, tea, chocolate or Coca-Cola like drinks) from 2 days before the first dose until 72 h after the last dose. Concomitant medications were not permitted throughout the study, unless required for treatment of adverse events (AEs). Blood samples (3 ml) for the determination of plasma concentrations of etamicastat and its metabolite BIA 5–961 were collected in lithium-heparin Vacuette® (Greiner Bio-One) tubes by means of an indwelling catheter or venipuncture at the following time points: pre dose, and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72, 96, and 120 h post dose in the young and elderly study, or at pre-dose and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48 and 72 h post last dose (Day 10) in the hypertensive patient study. After collection, blood samples were placed on ice and within 60 min after collection, the blood samples were centrifuged at approximately 1500 *g* and at 4 °C during 10 min. For the assay of etamicastat and metabolites, 4 aliquots of 250 μl each of the resulting plasma were withdrawn and placed into 2-ml cryotubes which were labeled, frozen and stored at <−70 °C until analysis. Safety was evaluated from reported AEs, physical examination, vital signs, digital 12-lead ECG, and clinical laboratory test results.

2.2.2.1. Cardiac evaluation after single ascending administration in young healthy humans. This was a single-center, entry-into-man, phase 1, double-blind, randomized, placebo-controlled study (trial registration EudraCT No. 2007-001181-33) in 10 sequential groups of 8 healthy male subjects each. In compliance with a randomization list generated by using computerized techniques, within each group 2 subjects were randomized to receive placebo and the remaining 6 to receive etamicastat. Etamicastat was administered as single oral doses of 2, 10, 20, 50, 100, 200, 400, 600, 900 or 1200 mg. Eligible volunteers were admitted to the unit 2 days prior (Day −2) to receiving the study medication (Day 1) and remained in the unit under clinical supervision for at least 72 h after dosing (Day 4). During admission, SBP and DBP and HR were recorded in supine position (after resting for at least 10 min) using a Dinamap® (GE Healthcare) monitor at the following times: Day −1 (the day prior to dosing) — time 0 (24 h before dosing) and 1, 2, 3, 4, 5, 6, 8, 10, 12 and 16 h after; Day 1 (dosing day) — pre-dose and 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, and 72 h post-dose. Orthostatic blood pressure and HR were recorded in standing position (after standing for approximately 2 min) at the following times: Day −1 — time 0 and 2, 4, 8, and 12 h after; Day 1 — pre-dose and 2, 4, 8, 12, and 24 h post-dose. 12-lead digital ECG were recorded on Day −1 at time 0 and 1, 2, 3, 4, 6, 8, 10, 12 and 16 h after, and on Day 1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, and 72 h post-dose. Digital ECGs were recorded after 10 min of rest, in triplicate (with an interval of 5 min, with a difference of at least 1 min between each of the 3 recordings). A general safety assessment and detailed pharmacokinetic evaluation was previously made available [21].

2.2.2.2. Cardiac evaluation after repeated oral administration in young and elderly healthy volunteers. This was an open-label, single-center, parallel-group study (trial registration EudraCT No. 2008-002127-82) in a group of 12 healthy young male subjects and a group of 12 healthy elderly male subjects. Participants were drawn from the study center's pool of volunteers. Volunteers eligible for participation were healthy male volunteers between 18 and 45 years (young group) or 65 years or more (elderly group), non-smokers or smokers of less than 10 cigarettes per day. Subjects were considered ineligible for participation if they: had been administered any investigational drug within 90 days, prescription drug within 30 days or over-the-counter drugs within 7 days before admission; showed to be prone to orthostatic hypotension, defined by a difference between supine SBP and standing SBP ≥20 mm Hg or a difference between supine DBP and standing DBP ≥10 mm Hg; or presented a ECG QTc interval reading ≥450 ms (young subjects) or ≥470 ms (elderly subjects). The trial consisted of a 100 mg etamicastat multiple-dose period during which participants received 100 mg etamicastat once-daily, for 7 days. A general safety assessment and detailed pharmacokinetic evaluation was previously made available [23].

2.2.2.3. Cardiac evaluation after repeated oral administration in hypertensive patients. This was a Phase IIa, double-blind, randomized, placebo-controlled study (trial registration EudraCT No. 2008-002789-69) investigating three dosage regimens of etamicastat (50, 100 or 200 mg, once-daily, for 10 days) in 3 groups of 8 hypertensive male patients aged between 18 and 45 years. Within each group, it was planned to randomize 2 subjects to receive placebo and the remaining 6 subjects to receive etamicastat. Randomization was performed by means of computerized techniques. Doses of 50, 100 and 200 mg of etamicastat were investigated in ascending order. The decision to proceed to the next dose depended on the tolerability assessment of the previous dose level. Each patient participated in the study for approximately 8 weeks. Participation included several screening evaluations within 4 weeks before admission to an inpatient period (Day −1 to Day 11), two ambulatory visits (Days 12 and 13) and a follow-up visit 7 to 10 days after the last administration. During the screening period, subjects received placebo once daily, in the morning, in a single-blind manner (subjects did not know they were receiving placebo, but the investigator was aware). Patients were admitted to the research facility in the morning of Day −1 (day before the first dose of investigational product) and remained inpatient until at least 24 h after the last dose, on Day 11. Then patients were discharged and instructed to

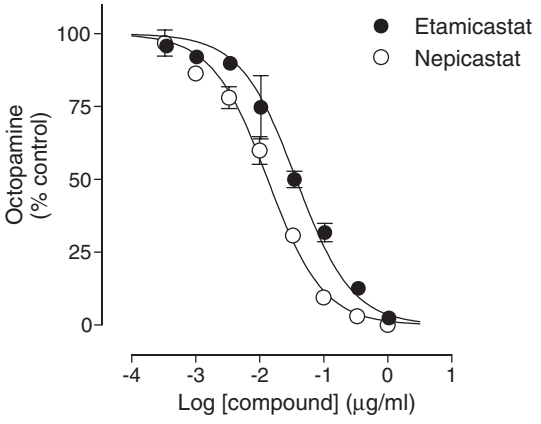

Fig. 2. Inhibition curve for inhibition of dopamine-β-hydroxylase activity by etamicastat and nepicatsat. Values are means ± SEM (n = 6).

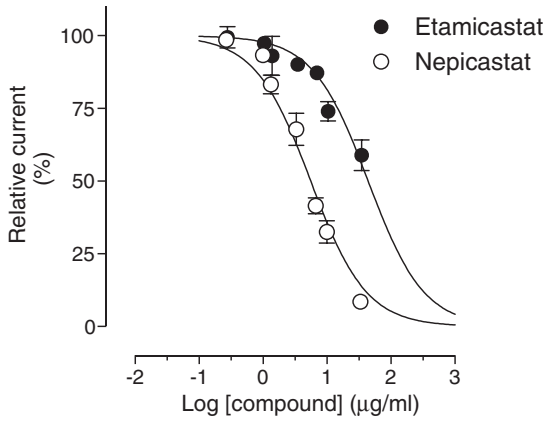

Fig. 3. Inhibition curve for blockade of hERG relative tail current by etamicastat. Values are means ± SEM (n = 3).

return for 48 h (Day 12) and 72 h (Day 13) post last dose assessments. During the inpatient period, from Day 1 to Day 10, participants received in a double-blind fashion either etamicastat 50, 100 or 200 mg or placebo, once daily, in the morning, with 250 ml water, under fasting conditions. Patients remained fasted until 4 h post-dose on Days 1 and 10, and until 1 h post-dose on Days 2 to 9. A general safety assessment and detailed pharmacokinetic evaluation was previously made available [20].

Safety was assessed from reported AEs, physical examination, vital signs, digital 12-lead ECG, and clinical laboratory test results. HIV and hepatitis B and C serology, and drugs of abuse screen were performed at screening; alcohol test was performed at screening and Day −1. Laboratory safety tests (hematology, blood chemistry and urinalysis) were performed at screening, Day −1, pre-dose on Days 5 and 10, Day 13 and follow-up visit.

Digital 12-lead ECG recordings were taken in supine position, after at least a 10-minute rest, at the screening visit and then at the following times: Day −1: time 0 (24 h before first dosing), and 1, 2, 3, 4, 6, 8, 10, 12 and 16 h after; Day 1 (day of first dosing): pre-dose, and 1, 2, 3, 4, 6, 8, 10, 12 and 16 h post-dose; from Day 2 to Day 9: pre-dose and 2 h post-dose; Day 10 (day of last dosing): pre-dose, and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48 and 72 h post-dose. The reported 12-lead ECG parameters included HR, PR interval, QRS interval duration and axis deviation, and QT interval. QT interval was corrected by the Fridericia's (QTcF) formula. The primary method of correction was QTcF. A manual reading of the digital ECGs was used to assess QT/QTc interval prolongation.

Safety standing blood pressure and HR measurements taken after the patient had been standing for 2 min were performed on Day −1, and at pre-dose and 2, 12 and 24 h post first (Day 1) and last dose (Day 10).

2.3. Assay of etamicastat and metabolites in plasma

Plasma concentrations of etamicastat and BIA 5–961 were determined using a validated method consisting of reversed phase liquid chromatography coupled with triple-stage quadrupole mass-spectrometric detection (LC/MS–MS), as previously described [26]. In brief, for the preparation of calibration samples, etamicastat and BIA 5–961 were dissolved in methanol to a final concentration of 250 µg/ml (plasma assay). For the quality control (QC) samples, a second set of stock solutions was prepared. For calibration and QC samples, working solutions in methanol were added to plasma using a ratio of 2/98 (v/v). For the preparation of the internal standard (ISTD) solution for the plasma assay, reference standard (BIA 5–1058, molecular formula $C_{21}H_{21}F_2N_3OS$) was dissolved in methanol to a concentration of 1000 µg/ml and then diluted in methanol to 5000 ng/ml; further dilutions to a final concentration of 50 ng/ml were done using acetonitrile/ethanol (50/50, v/v). For the preparation of the ISTD solution for the urine assays, reference standard was dissolved in methanol to a final concentration of 50 ng/ml. Plasma samples were vortexed and centrifuged for 20 min at approximately 3362 *g* and approximately 8 °C after unassisted thawing at room temperature. To an aliquot of 100 µl of plasma, 300 µl of acetonitrile/ethanol (50/50, v/v) containing 50 ng/ml of ISTD were added. After protein precipitation at room temperature, plasma samples were filtrated using a Captiva filter plate and an aliquot of 5 µl of the filtrate was injected onto the analytical column. To an aliquot of 20 µl of urine, 80 µl lithium-heparin plasma was added and were precipitated by 300 µl of methanol containing the ISTD. After protein precipitation at room temperature, urine samples were centrifuged for 20 min at 2773 *g* and 8 °C. An aliquot of 250 µl of the supernatant was transferred into an ultrafiltration filter plate and centrifuged for about 2 h at 4000 rpm (2773 *g*) and approximately 20 °C. An aliquot of 5 µl was injected onto the analytical column. The samples were stored in the autosampler tray at approximately 8 °C ± 5 °C.

The analytical equipment consisted of a Rheos 2000 pump (Flux Instruments, Basel, Switzerland), a SpeedROD, RP18e, 50–4.6 mm analytical column (Merck, Darmstadt, Germany), a ultra-low volume

Table 1
Mean arterial blood pressure (MABP), RR interval and QTcQ interval of the electrocardiogram following oral administration of vehicle, then etamicastat at 15, 45 and 90 mg/kg in the conscious *Cynomolgus* monkey monitored by telemetry.

Etamicastat (mg/kg)	Measurement times before and after administration (h)									
	−0.5	1	2	4	6	8	10	14	18	22
MABP (mm Hg)										
0	80 ± 3	82 ± 6	78 ± 4	79 ± 5	75 ± 4	78 ± 5	60 ± 6	64 ± 4	65 ± 5	79 ± 6
15 mg/kg	79 ± 4	78 ± 4	88 ± 8	74 ± 2	74 ± 6	77 ± 4	59 ± 4	64 ± 3	65 ± 3	76 ± 6
45 mg/kg	81 ± 7	83 ± 6	89 ± 7	74 ± 8	73 ± 6	76 ± 7	58 ± 6	59 ± 5	65 ± 6	74 ± 6
90 mg/kg	83 ± 9	80 ± 7	85 ± 10	77 ± 6	72 ± 6	76 ± 7	61 ± 7	64 ± 6	64 ± 5	68 ± 7*
RR interval (ms)										
0	405 ± 40	430 ± 6	461 ± 32	420 ± 16	412 ± 21	436 ± 14	572 ± 10	603 ± 38	613 ± 24	412 ± 39
15 mg/kg	421 ± 22	455 ± 9	417 ± 17	465 ± 19	429 ± 26	446 ± 11	592 ± 31	621 ± 51	602 ± 30	426 ± 18
45 mg/kg	395 ± 30	422 ± 34	431 ± 27	454 ± 28	445 ± 22	438 ± 16	596 ± 20	666 ± 26	588 ± 4	407 ± 20
90 mg/kg	385 ± 18	428 ± 11	398 ± 28	423 ± 18	470 ± 28	456 ± 4	552 ± 20	588 ± 43	561 ± 37	464 ± 16
QTcQ interval (ms)										
0	250 ± 2	242 ± 7	248 ± 8	247 ± 7	251 ± 8	245 ± 5	251 ± 5	254 ± 7	253 ± 5	247 ± 6
15 mg/kg	242 ± 5	248 ± 4	255 ± 3	246 ± 6	246 ± 5	241 ± 4	245 ± 9	236 ± 5	245 ± 12	266 ± 15
45 mg/kg	253 ± 9	252 ± 11	253 ± 8	251 ± 11	247 ± 9	243 ± 11	248 ± 13	252 ± 10	251 ± 13	259 ± 5
90 mg/kg	249 ± 6	247 ± 5	252 ± 5	253 ± 3	241 ± 6	239 ± 2	257 ± 10	251 ± 9	254 ± 10	242 ± 6

The placebo and etamicastat were administered orally, by capsule, at time 0. Values are mean ± SEM of results obtained from 4 animals.
* Denotes $P < 0.05$ etamicastat vs vehicle.

precolumn filter, 2 μm (Upchurch Scientific Inc, Oak Harbor, WA, USA), a TSQ Quantum mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), and a HTS PAL autosampler (CTC Analytics AG, Zurich, Switzerland). The MS detector was operated in positive ion mode with mass transitions for etamicastat, BIA 5–961 and the ISTD of respectively 283.0 amu, 127.0 amu and 120.0 amu. Column temperature was 50 °C. The mobile phases used water containing 0.5% formic acid (phase A), water containing 1.0% formic acid (phase B), acetonitrile containing 1.0% formic acid (phase C) and acetonitrile containing 0.01% formic acid (phase D). Calibration curves over the nominal concentration range 5–5000 ng/ml for plasma assays and a set of quality control (QC) samples were analyzed with each batch of study samples. The QC samples were prepared in duplicates at three concentration levels (low, medium and high). The analytical method was demonstrated to be precise and accurate. The descriptive statistics of the QC samples showed that the overall imprecision of the method, measured by the inter-batch coefficient of variation, was ≤7.1% for etamicastat and ≤7.5% for BIA 5–961. The inter-batch accuracy ranged from 101.5% to 105.3% for etamicastat and 101.0% to 105.3% for BIA 5–961. The lower limit of quantification of the assay (LLOQ) was 5 ng/ml in plasma, for both compounds.

2.4. Statistical analysis

Reported values are expressed as means ± standard error of the mean (SEM) or means with confidence intervals (CI). Statistical analysis was performed using the Dynamic Microsystem software GB-Stat version 6.5. For in vitro (hERG) study, multisample analysis (ANOVA followed by Dunnett's test) was performed to test statistical significance of all concentrations tested. For in vivo (telemetry) studies, intra-group comparison was performed using an one-way analysis of variance (time) with repeated measures at each time, followed by Dunnett's *t*

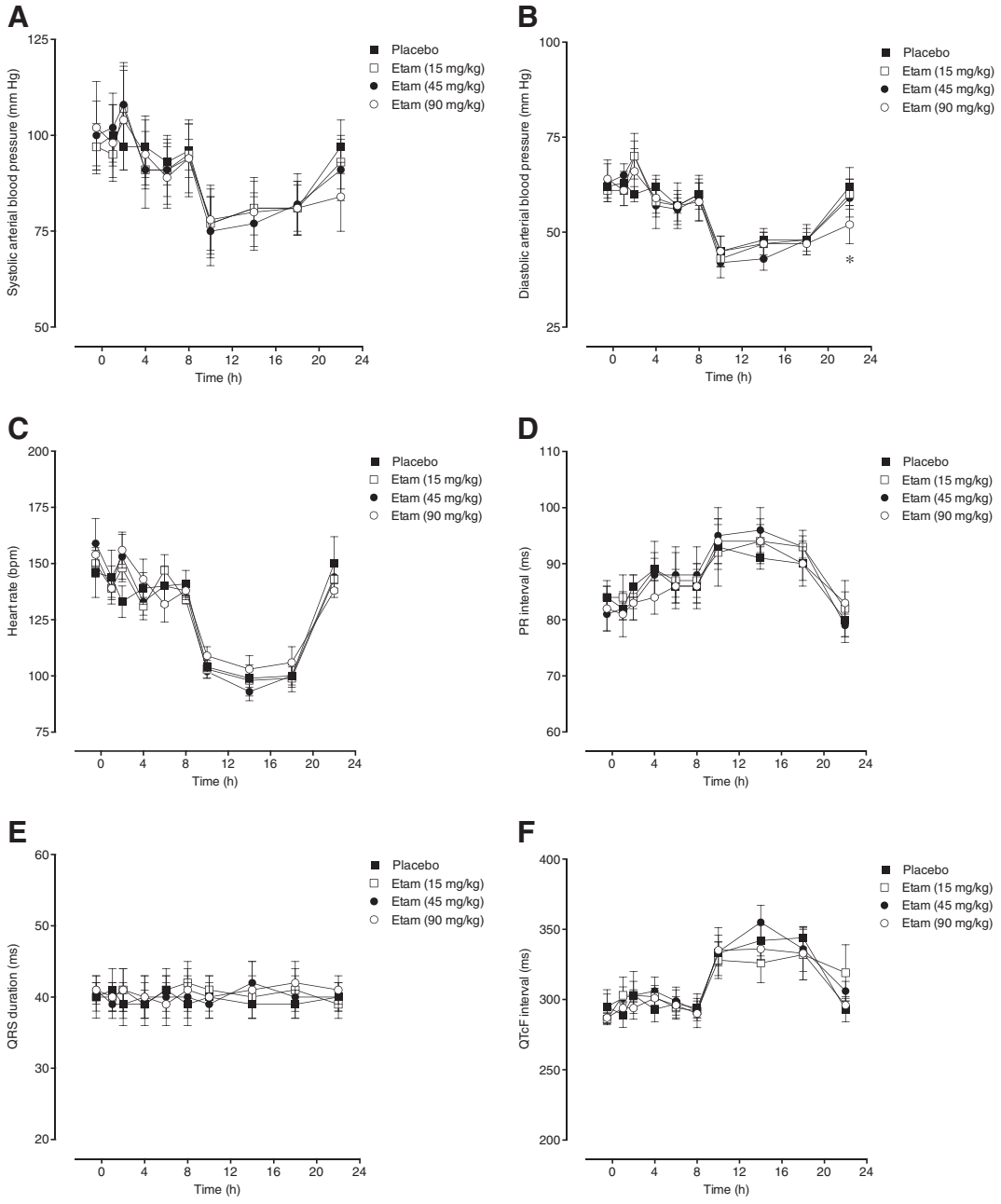

Fig. 4. Effects of placebo and etamicastat on arterial blood pressure, heart rate, and the main parameters of the electrocardiogram (PR interval, QRS duration and QTcF interval) following oral (p.o. capsule) administration in the conscious male *Cynomolgus* primate monitored by telemetry. Symbols represent mean ± SEM values of values of 4 animals per group.

Table 2
ECG parameters in female and male *Cynomolgus* monkeys before and following oral administration of vehicle and etamicastat at 15 and 45 mg/kg.

			Heart rate (bpm)	P amplitude (mV)	P duration (ms)	P–Q interval (ms)	QRS interval (ms)	Q–T interval (ms)
Pre-test								
Vehicle (0 mg/kg/day)		Females	243 ± 20	0.09 ± 0.02	41 ± 2	58 ± 4	50 ± 0	180 ± 11
		Males	227 ± 46	0.10 ± 0.00	43 ± 5	64 ± 7	52 ± 4	180 ± 21
Etamicastat (15 mg/kg/day)		Females	250 ± 8	0.09 ± 0.01	46 ± 5	60 ± 0	50 ± 0	173 ± 10
		Males	258 ± 17	0.10 ± 0.01	44 ± 5	60 ± 8	53 ± 5	170 ± 8
Etamicastat (45 mg/kg/day)		Females	223 ± 23	0.10 ± 0.00	43 ± 5	63 ± 9	55 ± 6*	185 ± 13
		Males	263 ± 24	0.06 ± 0.03*	44 ± 3	63 ± 5	50 ± 0	166 ± 8
Week 1								
Vehicle (0 mg/kg/day)	before dosing	Females	237 ± 26	0.09 ± 0.03	40 ± 0	60 ± 0	50 ± 0	183 ± 15
	1 h after dosing		258 ± 21	0.08 ± 0.02	40 ± 0	59 ± 2	51 ± 2	178 ± 12
	before dosing	Males	238 ± 42	0.11 ± 0.02	44 ± 4	66 ± 8	57 ± 5	183 ± 14
	1 h after dosing		238 ± 31	0.10 ± 0.03	46 ± 5	67 ± 8	53 ± 5	180 ± 11
Etamicastat (15 mg/kg/day)	before dosing	Females	245 ± 6	0.09 ± 0.01	41 ± 3	63 ± 3	50 ± 0	183 ± 5
	1 h after dosing		233 ± 21	0.08 ± 0.05	43 ± 3	65 ± 4	53 ± 5	185 ± 10
	before dosing	Males	240 ± 41	0.08 ± 0.03	43 ± 5	56 ± 5	53 ± 5	176 ± 11
	1 h after dosing		225 ± 31	0.09 ± 0.01	40 ± 0	60 ± 0	53 ± 5	180 ± 12
Etamicastat (45 mg/kg/day)	before dosing	Females	225 ± 39	0.10 ± 0.01	48 ± 10	68 ± 10	53 ± 5	190 ± 18
	1 h after dosing		208 ± 30*	0.09 ± 0.03	43 ± 5	64 ± 8	50 ± 0	198 ± 17
	before dosing	Males	220 ± 40	0.09 ± 0.01	40 ± 0	63 ± 5	53 ± 5	190 ± 20
	1 h after dosing		233 ± 22	0.09 ± 0.03	43 ± 5	63 ± 5	55 ± 6	193 ± 15
Week 13								
Vehicle (0 mg/kg/day)	before dosing	Females	252 ± 18	0.09 ± 0.02	40 ± 0	58 ± 4	58 ± 4	177 ± 12
	1 h after dosing		230 ± 24	0.07 ± 0.02	40 ± 0	58 ± 4	58 ± 4	187 ± 21
	before dosing	Males	246 ± 36	0.12 ± 0.07	42 ± 4	61 ± 5	60 ± 0	183 ± 15
	1 h after dosing		210 ± 44	0.10 ± 0.03	45 ± 5	67 ± 8	60 ± 0	197 ± 26
Etamicastat (15 mg/kg/day)	before dosing	Females	240 ± 24	0.10 ± 0.01	41 ± 3	59 ± 3	60 ± 0	190 ± 12
	1 h after dosing		218 ± 15	0.10 ± 0.01	44 ± 5	63 ± 5	60 ± 0	200 ± 0
	before dosing	Males	250 ± 22	0.08 ± 0.03	40 ± 0	59 ± 3	60 ± 0	185 ± 13
	1 h after dosing		225 ± 44	0.08 ± 0.03	40 ± 0	60 ± 0	60 ± 0	193 ± 22
Etamicastat (45 mg/kg/day)	before dosing	Females	213 ± 49	0.11 ± 0.02	40 ± 0	59 ± 3	63 ± 5	195 ± 31
	1 h after dosing		203 ± 25	0.10 ± 0.01	43 ± 5	68 ± 10#	63 ± 5	203 ± 17
	before dosing	Males	253 ± 38	0.10 ± 0.00	43 ± 5	59 ± 3	60 ± 0	180 ± 14
	1 h after dosing		243 ± 38	0.08 ± 0.02	43 ± 5	63 ± 5	60 ± 0	183 ± 21

Values are means ± SEM. Significantly different from corresponding values in vehicle treated animals (*$P < 0.05$) following Dunnett test.
Significantly different from corresponding values before dosing 1 (#$P < 0.05$) following Dunnett test.

test in case of significant time effect, to compare each time value with the T0 values (i.e. basal value before each treatment). Inter-group statistical analysis was also performed using a two-way analysis of variance (group, time) with repeated measures at each time, followed by a one-way analysis of variance (group) at each time in case of significant group × time interaction. The analysis was completed by Dunnett's t tests where the group effect was significant. In case of data considered invalid or missing data, the retained value was taken 5 min before or after the theoretical time, or was represented by the mean of data taken 5 or 10 min before and 5 or 10 min after the theoretical time.

2.5. Drugs

Etamicastat, BIA 5–961 and reference standard (BIA 5–1058) were supplied by BIAL (Laboratory of Chemistry, S. Mamede do Coronado, Portugal).

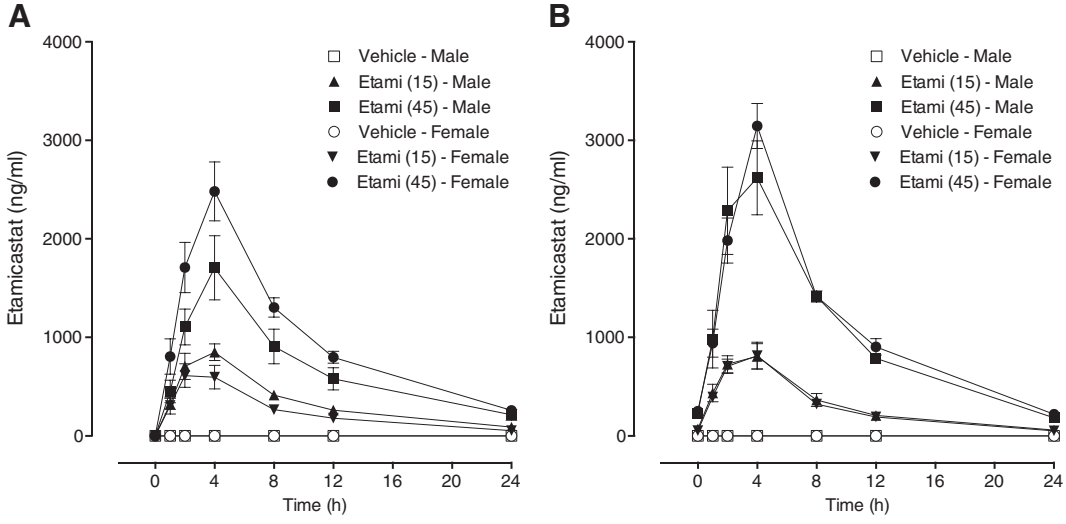

Fig. 5. Plasma concentration-time profiles of etamicastat after single and 91 once daily administrations by oral gavage of 15 and 45 mg/kg/day etamicastat in male and female *Cynomolgus* monkeys. Symbols represent mean ± SEM values of values of 4 animals per group.

Table 3
Pharmacokinetic parameters of etamicastat in female and male *Cynomolgus* monkeys after single and 91 once daily administrations of etamicastat by oral gavage.

		Day 1			Day 91		
Dose (mg/kg)		15	45	Ratio	15	45	Ratio
Females							
C_{max}	(ng/ml)	686.0 ± 118.8	2497.5 ± 284.3	4.3 ± 1.4	868.8 ± 122.3	3145.0 ± 228.5	3.9 ± 0.7
t_{max}	(h)	2.5 ± 0.5	3.5 ± 0.5	1.6 ± 0.4	3.5 ± 0.5	4.0 ± 0.0	1.3 ± 0.3
AUC_{0-24}	(ng·h/ml)	5591.8 ± 1014.5	23022.0 ± 1898.3	4.9 ± 1.5	6688.0 ± 1016.4	26180.8 ± 943.0	4.2 ± 0.7
$AUC_{0-\infty}$	(ng·h/ml)	6136.0 ± 1130.7	25678.3 ± 2336.9	5.1 ± 1.6	7142.0 ± 1150.3	28024.5 ± 989.9	4.3 ± 0.7
$t_{½}$	(h)	6.9 ± 0.3	6.9 ± 0.4	1.0 ± 0.1	6.0 ± 0.3	5.8 ± 0.1	1.0 ± 0.0
Males							
C_{max}	(ng/ml)	935.5 ± 71.4	1875.0 ± 179.8	2.0 ± 0.0	834.3 ± 101.7	2895.0 ± 306.9	3.5 ± 0.2
t_{max}	(h)	3.0 ± 0.6	3.5 ± 0.5	1.3 ± 0.3	3.5 ± 0.5	3.0 ± 0.6	0.9 ± 0.1
AUC_{0-24}	(ng·h/ml)	7902.0 ± 339.7	16137.8 ± 2294.1	2.0 ± 0.2	7125.0 ± 1159.0	24168.8 ± 1426.4	3.7 ± 0.7
$AUC_{0-\infty}$	(ng·h/ml)	8873.5 ± 358.0	18549.3 ± 2787.9	2.1 ± 0.3	7671.3 ± 1232.2	25662.8 ± 1420.6	3.7 ± 0.6
$t_{½}$	(h)	7.4 ± 0.4	7.4 ± 0.7	1.0 ± 0.1	6.4 ± 0.4	5.6 ± 0.4	0.9 ± 0.1

Values are means ± SEM of 4 animals per group.

3. Results

3.1. In vitro studies

3.1.1. DβH activity

The experimental conditions, for evaluating the conversion of tyramine into octopamine by human SK-N-SH cell homogenates, were previously optimized with time and protein dependency experiments (data not shown). The formation of octopamine was dependent on the incubation time up to 1 h ($r^2 = 0.998$) and on protein amount up to 125 μg total protein per assay ($r^2 = 0.995$). With 75 μg total protein and 45 min incubation time, octopamine was formed by SK-N-SH cell homogenates with a K_m value of 9 (CI, 6; 13) mM for tyramine and a V_{max} of 1725 ± 76 nmol/mg protein/h. Under these optimized conditions, etamicastat and nepicastat inhibited SK-N-SH DβH activity in a concentration dependent manner (Fig. 2) with IC_{50} values (in ng/ml) of 37.2 (CI, 30.7; 44.9) and 13.3 (CI, 11.6; 15.4), respectively.

3.1.2. hERG K^+ channel

Etamicastat and nepicastat produced reductions of hERG current amplitude (Fig. 3) with IC_{50} values (μg/ml) of 44.0 (CI, 34.8; 55.7) and 5.6 (CI, 4.7; 6.5), respectively. In comparison, the positive control substance E-4031 at 100 nM markedly blocked the hERG tail current (8.88 ± 1.65% relative tail current). The observed inhibition of hERG tail currents by E-4031 was in line with its known pharmacological profile [27].

3.2. In vivo studies

3.2.1. Cynomolgus primates monitored by telemetry

The effects of etamicastat (15, 45 and 90 mg/kg) on arterial blood pressure, heart rate and the main parameters of the electrocardiogram were evaluated following oral (p.o. capsule) administration in the conscious male *Cynomolgus* primate monitored by telemetry (Table 1 and Fig. 4). The doses of etamicastat were based on the results from a maximum tolerated dose (MTD) study in *Cynomolgus* monkeys in which the MTD was established as 120 mg/kg/day (BIAL data on file). There was 1 treatment group of 4 primates following a 4 × 4 Latin square design. There was a washout period of 1 week between each treatment. Animals were dosed at approximately the same time each day. Venous blood samples (approximately 1 ml) for determination of test substance in plasma were taken prior to dosing and at 2 and 4 h after the start of dose administration, or as close as was reasonably practicable to these time points. Loose feces were noted in the cage of 1 animal following the administration of 90 mg/kg etamicastat. Although this was an isolated incidence in a single animal, it was observed following the highest dose of test substance and therefore may be related to etamicastat administration.

Arterial blood pressure (SBP, DBP and MBP) was generally unaffected following the administration of etamicastat at any of the doses examined with the exception of a non-statistically significant increase 2 h following the administration of 15, 45 and 90 mg/kg etamicastat (Table 1 and Fig. 4). However, the magnitude of these changes were small, reflecting increases from pre-dose baseline in MBP of 12.1 ± 8.3%,

Table 4
Summary statistics of demographic and baseline characteristics of study participating human subjects.

Study	Etamicastat group (n)	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	HR (bpm)
Single ascending dose study	Placebo (20)	30.8 (19–44)	178.2 (166–195)	72 (61–92)	22.7 (20.4–27.1)	106 (86–116)	61 (46–73)	54 (38–70)
	2 mg (6)	32.0 (21–38)	182.5 (173–194)	82 (68–97)	24.7 (18.1–29.9)	116 (102–127)	68 (60–80)	60 (44–70)
	10 mg (6)	30.3 (20–44)	182.2 (175–191)	79 (72–90)	23.7 (20.8–25.8)	107 (96–113)	62 (57–70)	48 (40–55)
	20 mg (6)	26.8 (19–32)	178.8 (170–186)	66 (56–75)	20.6 (18.5–21.7)	99 (88–107)	56 (53–63)	52 (44–62)
	50 mg (6)	28.8 (20–36)	175.7 (173–179)	70 (62–81)	22.5 (20.2–25.3)	111 (100–120)	58 (47–66)	57 (45–80)
	100 mg (6)	29.8 (19–40)	175.5 (171–183)	68 (60–80)	21.9 (18.8–25.8)	104 (95–111)	55 (50–58)	56 (53–61)
	200 mg (6)	30.7 (20–42)	176.3 (169–191)	72 (63–83)	23.2 (21.7–25.1)	109 (96–144)	60 (56–74)	58 (46–70)
	400 mg (6)	28.2 (25–32)	173.2 (167–181)	70 (60–79)	23.5 (21.0–26.9)	119 (102–146)	60 (53–66)	59 (47–80)
	600 mg (6)	31.3 (22–41)	174.7 (161–183)	74 (63–84)	24.3 (22.7–26.8)	114 (97–138)	59 (51–69)	74 (63–84)
	900 mg (6)	37.7 (22–43)	172.0 (164–178)	64 (852–73)	21.5 (19.0–25.6)	107 (102–117)	61 (46–68)	60 (48–72)
	1200 mg (6)	31.7 (19–38)	179.0 (172–184)	77 (63–88)	23.8 (21.3–26.5)	111 (99–123)	57 (49–61)	61 (57–66)
Young & elderly study	100 mg Young group (13)	32.6 (18–44)	175.8 (162–185)	79.0 (57.0–102)	25.8 (20.9–34.4)	117 (103–137)	61 (50–75)	58 (47–70)
	100 mg Elderly group (12)	69.3 (65–75)	166.9 (160–179)	69.2 (52–86)	25.2 (19.8–29.0)	115 (98–143)	64 (56–68)	66 (52–76)
Hypertensive patients study	Placebo (5)	59.6 (53–64)	169 (162–180)	83.2 (74–102)	29.0 (25.0–33.5)	160.6 (123–174)	92.6 (89–99)	70.7 (55–82)
	50 mg (5)	55.8 (51–61)	177 (169–184)	84.3 (64–101)	26.8 (22.4–32.2)	150.2 (141–161)	94.0 (84–99)	64.5 (56–68)
	100 mg (6)	56.5 (49–64)	176 (168–181)	84.8 (73–101)	27.3 (24.1–30.8)	164.8 (151–187)	96.8 (86–109)	63.8 (55–72)
	200 mg (6)	59.8 (58–61)	171 (165–179)	83.3 (71–105)	28.3 (24.6–32.8)	147.2 (129–163)	87.2 (78–93)	60.0 (49–72)

BMI, body mass index; SBP, supine systolic blood pressure; DBP, supine diastolic blood pressure; HR; heart rate beats per min.
Values are means with range values in parenthesis.

10.2 ± 5.2% and 2.3 ± 4.0% following 15, 45 and 90 mg/kg etamicastat administration, respectively, in comparison to a decrease of 2.0 ± 1.5% at this time following placebo treatment and were mainly due to an increase in the arterial blood pressure of a single animal (Table 1). Furthermore, no dose-dependency was evident, consequently, the observed increases were not considered to be etamicastat related. In addition, a decrease in arterial blood pressure was evident 22 h following the administration of 90 mg/kg etamicastat ($P < 0.05$ for DBP and MBP) (Fig. 4B). At this time, arterial blood pressure was maintained at similar values to those observed throughout the dark phase of the animals' light/dark cycle, was within the range of values observed following placebo treatment and as this decrease was noted some considerable time following an oral dose, it was not considered to be related to the administration of etamicastat.

HR was noted to be slightly elevated, although not significantly, in comparison to placebo at 2 h following the administration of 15, 45 and 90 mg/kg etamicastat (Fig. 4C). However, when compared to predose baseline values, this increase was no longer evident. HR was otherwise unaffected following the administration of etamicastat with the exception of a tendency for this parameter to be decreased 14 h

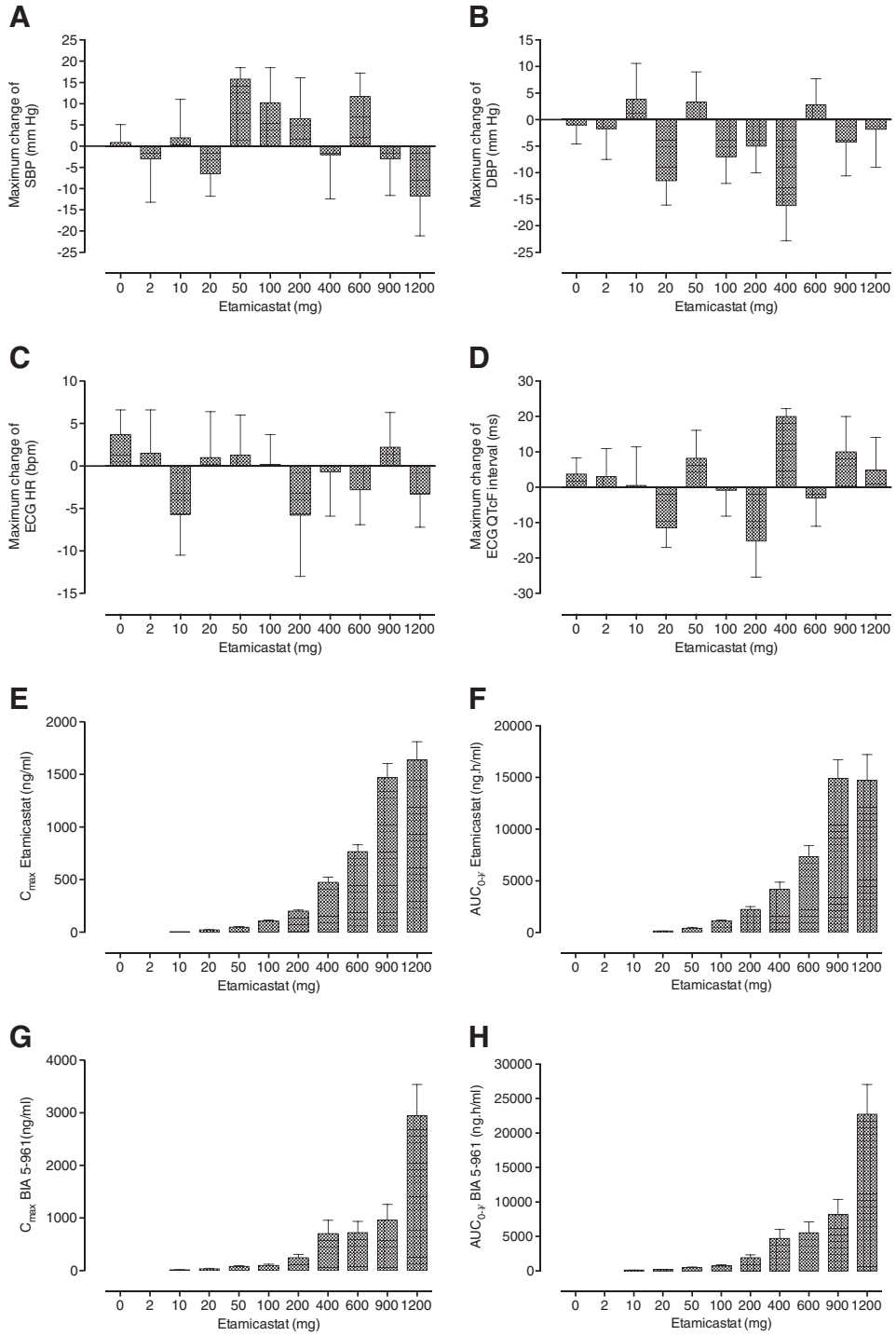

Fig. 6. Mean change from baseline of supine systolic blood pressure, diastolic blood pressure, heart rate, PR interval, QTcF interval, C_{max} and $AUC_{0-\infty}$ etamicastat and BIA 5–961 in young healthy subjects after single once-daily administration of etamicastat. Symbols represent mean ± SEM values of 6 subjects per group. Significantly different from corresponding values before dosing ($^{\#}P < 0.05$) following Dunnett test.

following the administration of 45 mg/kg etamicastat. As this was an isolated incidence, not observed following the highest dose of etamicastat and was noted some time following an oral administration, it was not considered to be etamicastat related. RR interval was generally unaffected following the administration of etamicastat with the exception of changes concomitant with those observed in HR (Table 1). Similarly, and for reasons discussed for HR, these changes were not considered to be related to the administration of etamicastat. PR interval and QRS duration were unaffected following the administration of etamicastat at any of the doses examined (Fig. 4E).

There was no marked effect on QT interval following the administration of 15, 45 or 90 mg/kg etamicastat with the exception of a prolongation 14 h following the administration of 45 mg/kg etamicastat, corresponding with the changes observed in heart rate and RR interval at this time. When the QT interval was corrected for changes in HR/RR interval by calculation of the QTcF interval (Fig. 4F), this prolongation, although not significant, was still observed; however, when corrected using the individual animal specific correction formula, QTcQ, it was no longer apparent (Table 1). A slight shortening of the QTcQ interval was noted at 14 h following the administration of 15 mg/kg etamicastat when compared to placebo (Table 1). However, values remained unchanged when compared to pre-dose baseline; therefore, this was not considered to be a drug-related effect.

Mean plasma concentrations of etamicastat at 2 and 4 h were 610 ± 160 and 646 ± 68, 771 ± 304 and 1968 ± 356, and 1055 ± 76.5 and 1926 ± 592 ng/ml at the dose levels of 15, 45 and 90 mg/kg, respectively.

3.2.2. Cardiac evaluation after repeated oral administration in Cynomolgus primates

In this set of experiments, cumulative effects of etamicastat were assessed in the female and male *Cynomolgus* monkeys following oral administration by gastric gavage once daily for at least 91 days. Animals received etamicastat at 0, 15 or 45 mg/kg/day; the control group only received distilled water as vehicle. Diarrhea and soft feces were frequently observed in groups receiving 15 or 45 mg/kg/day etamicastat. In all groups, no treatment-related ophthalmoscopic alteration was recorded. No effects resulting from treatment with etamicastat were observed on hematology, biochemistry or urinalysis. There were no treatment-related findings in the electrocardiograms (heart rate, P amplitude, P duration, P–Q interval, QRS interval and Q–T interval) (Table 2). Occasional incidences of incomplete right bundle branch block were observed in three vehicle-treated animals and sinus arrhythmia was observed in two vehicle-treated and one animal receiving 45 mg/kg/day etamicastat. These were considered not related to treatment with etamicastat as they were seen at similar incidences in both groups and also observed during acclimatization.

Plasma samples were obtained on Day 1 and in Day 91 of the treatment period at 0 (pre-dose) 1, 2, 4, 8, 12 and 24 h after the administration of etamicastat. All samples were analyzed for concentrations of etamicastat. All plasma concentrations of etamicastat in male and female monkeys receiving only vehicle were below the limit of quantification (5 ng/ml). All animals in the treated dose groups were consistently exposed to etamicastat after single and repeated oral (gavage) administration. The number of quantifiable samples was sufficient to calculate AUC_{0-t}, t_{max}, C_{max}, and $t_{½,z}$, from the concentration versus time profiles (Fig. 5) and to investigate the effects of dose, gender and single versus repeated administration on exposure. Maximum plasma concentrations (C_{max}) of etamicastat in female and male monkeys in all groups were reached 1 to 8 h after administration (Table 3). After t_{max}, plasma concentrations declined rapidly (Fig. 5) with half-lives ranging between 5.0 and 8.4 h (Table 3). Throughout all groups, mean half-lives seemed to be dose, gender or time independent. After single administration of etamicastat from 15 to 45 mg/kg/day with 3.0-fold dose increase, exposure to etamicastat in males increased less than expected the given dose increase: AUC_{0-t} increased by a factor of 2.0 ± 0.6. A similar increase was observed for C_{max} (ratio of 2.0 ± 0.5). In females, from 15 to 45 mg/kg/day AUC_{0-t} and C_{max} increased more than dose-proportionally (ratios of 4.1 ± 1.6 and 3.6 ± 1.5, respectively). After 13 weeks of repeated treatment, from 15 to 45 mg/kg/day with 3.0-fold dose increase, exposure to etamicastat in both gender increased more than dose-proportionally: AUC_{0-t} and C_{max} increased by a factor of 3.9 ± 1.2 and 3.6 ± 1.1 for females and 3.4 ± 1.2 to 3.5 ± 1.1 for males, respectively. Throughout all groups, half-lives seemed to be dose-independent (half-lives ratios of 0.9 ± 0.2 to 1.0 ± 0.1).

3.2.3. Cardiac evaluation after single ascending administration in young healthy humans

A total of 128 male subjects were screened in order to include 80 subjects in 10 successive groups of 8 subjects each. All 80 included subjects were randomized and completed the study. There was no subject withdrawal or premature discontinuation. No relevant differences in

Table 5
Summary pharmacokinetic parameters of etamicastat.

Study	Etamicastat group (n)	C_{max} (ng/ml)	C_{min} (ng/ml)	t_{max} (h)	AUC_{0-t} (ng·h/ml)	$AUC_{0-\infty}$ (ng·h/ml)	$t_{1/2}$ (h)
Single ascending dose study	Placebo (20)	ND	ND	ND	ND	ND	ND
	2 mg (6)	ND	ND	ND	ND	ND	ND
	10 mg (6)	6 ± 1	ND	2.0 (1.0–2.0)	4 ± 2	ND	ND
	20 mg (6)	23 ± 8	6.1 ± 1.5	1.0 (0.5–2.0)	84 ± 36	148 ± 25	6.6 ± 1.6
	50 mg (6)	49 ± 13	6.1 ± 1.2	1.0 (0.5–2.0)	304 ± 181	421 ± 238	12.6 ± 6.0
	100 mg (6)	106 ± 24	6.3 ± 0.9	1.5 (1.0–3.0)	957 ± 212	1134 ± 250	19.7 ± 5.7
	200 mg (6)	202 ± 24	7.0 ± 1.5	1.0 (1.0–3.0)	2026 ± 663	2230 ± 701	19.8 ± 3.3
	400 mg (6)	475 ± 119	9.9 ± 2.5	2.0 (1.0–2.1)	3914 ± 1637	4171 ± 1733	17.3 ± 3.7
	600 mg (6)	766 ± 161	10.5 ± 4.9	3.0 (1.0–5.0)	7111 ± 2480	7355 ± 2566	15.7 ± 1.6
	900 mg (6)	1469 ± 327	26.6 ± 9.4	3.0 (1.0–5.0)	14197 ± 4231	14908 ± 4428	18.8 ± 1.5
	1200 mg (6)	1638 ± 423	26.7 ± 17.1	2.5 (1.0–3.0)	13997 ± 5687	14707 ± 6147	18.2 ± 1.6
Young & Elderly study[a]	100 mg Young group (13)	105 ± 37	15.0 ± 11.1	1.5 (1.0–4.0)	1142 ± 692	1497 ± 825	17.3 ± 5.8
	100 mg Elderly group (12)	124 ± 33	18.5 ± 11.9	1.0 (0.5–5.0)	1666 ± 1026	2199 ± 1262	28.1 ± 12.2
Hypertensive study[b]	Placebo (5)	ND	ND	ND	ND	ND	ND
	50 mg (5)	62 ± 24	12.7 ± 2.0	1.0 (0.5–2.0)	769 ± 456	1123 ± 676	18.5 ± 9.5
	100 mg (6)	168 ± 56	14.3 ± 3.2	1.0 (0.5–2.0)	2387 ± 1366	2906 ± 1603	24.6 ± 9.2
	200 mg (6)	277 ± 118	16.5 ± 5.9	1.0 (1.0–2.0)	3633 ± 1845	4307 ± 2050	28.0 ± 3.1

ND = not detected.
Values are means ± SD or means with range values in parenthesis.
[a] PK parameters refer to last day of once-daily administration = 7 days.
[b] PK parameters refer to last day of once-daily administration = 10 days.

demographic characteristics were found between the treatment groups (Table 4). Fig. 6 displays the maximum change from baseline of supine SBP, DBP, HR and QTcF interval in young healthy subjects after once-daily single administration of placebo or etamicastat (2 to 1200 mg). There was no clinically significant change in SBP, DBP, HR and QTcF interval, though important variability was observed. A total of 18 AEs were reported in 13 subjects. There was no serious AE and no TEAE required the withdrawal of a subject. All TEAEs were mild to moderate in

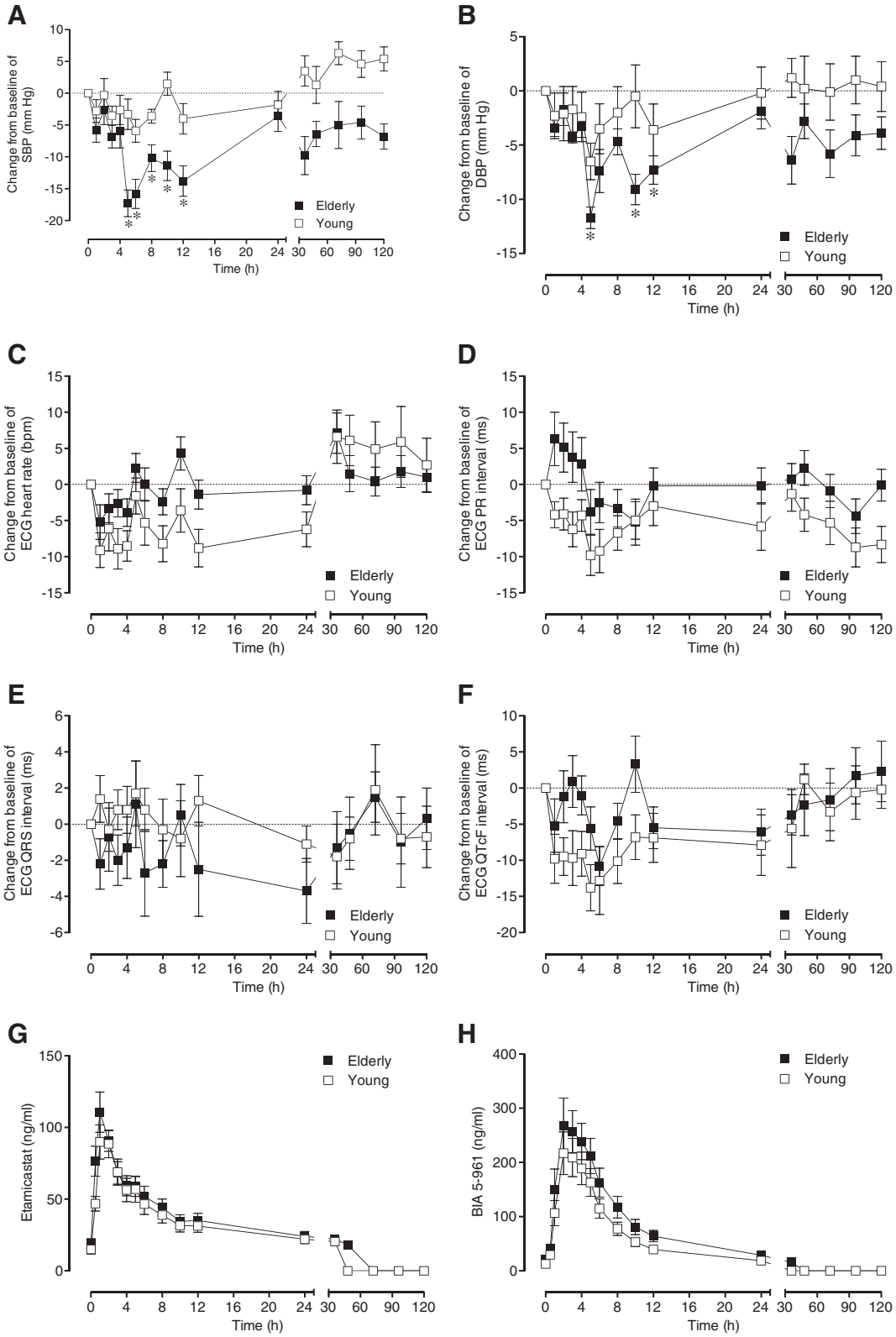

Fig. 7. Mean change from baseline of supine systolic blood pressure, diastolic blood pressure, heart rate, PR interval, QRS duration and QTcF interval, and concentration time profiles of etamicastat and BIA 5–961 in young and elderly healthy subjects after once-daily administration of etamicastat (100 mg/day) for 7 days. Symbols represent mean ± SEM values of 12 and 13 subjects per group. Significantly different from corresponding values before dosing (*$P < 0.05$) following Dunnett test.

intensity. Etamicastat was well tolerated at all the investigated dose levels. Mean C_{max} and area under the plasma concentrations versus time curve from time 0 to infinity ($AUC_{0-\infty}$) etamicastat plasma levels following single doses of 2, 10, 20, 50, 100, 200, 400, 600, 900 and 1200 mg of etamicastat are displayed in Fig. 6. Etamicastat plasma concentrations could not be detected at the 2 mg dose level and were detected in only 3 subjects and at very few sampling time-points at the 10 mg dose level. BIA 5–961 plasma concentrations could only be detected in two subjects at the 2 mg dose, and therefore mean pharmacokinetic parameters are only depicted for etamicastat 10 mg and above doses (Fig. 6). The corresponding pharmacokinetic parameters are presented in Table 5. Although systemic exposure appeared to increase

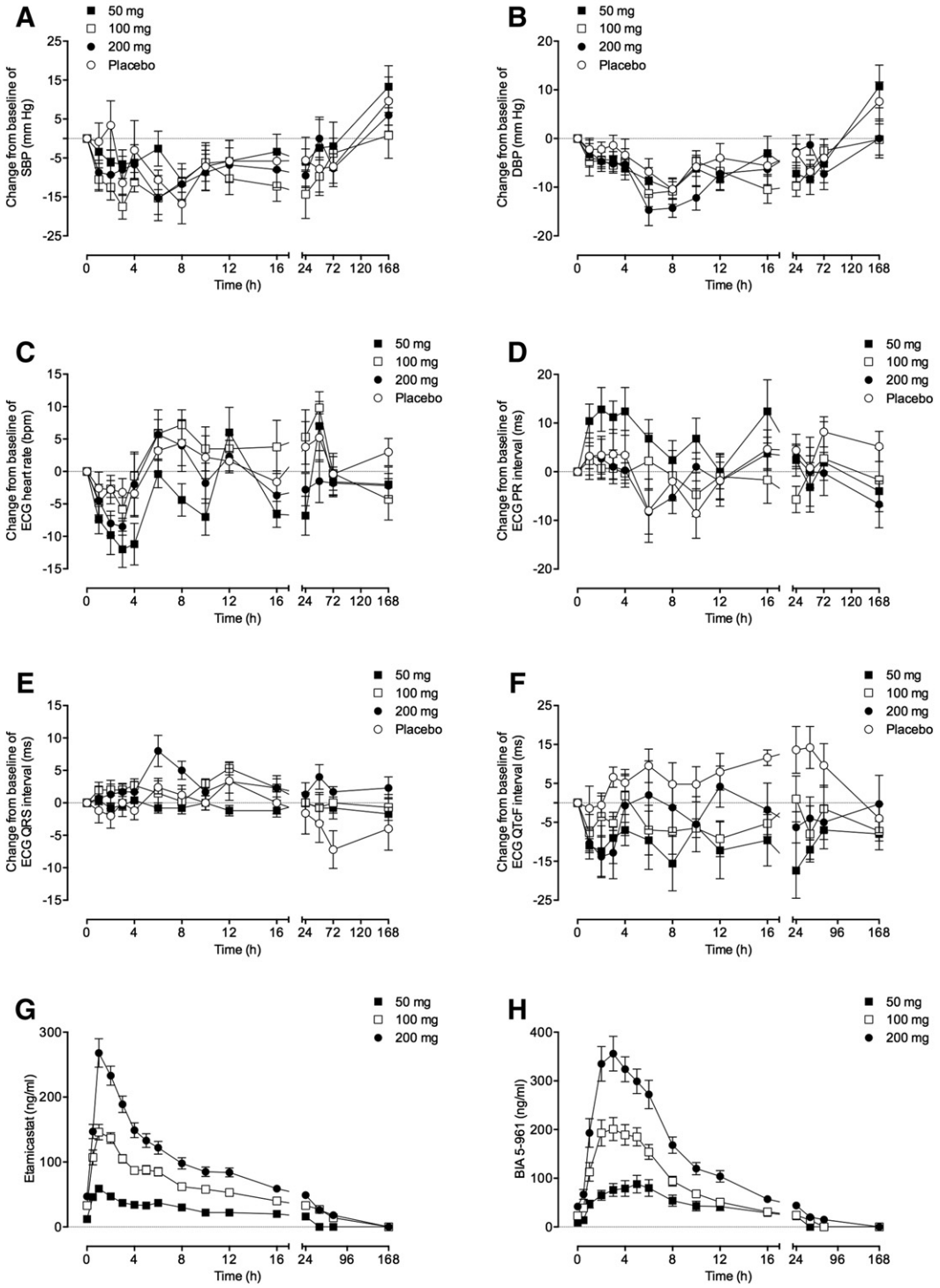

Fig. 8. Mean change from baseline of supine systolic blood pressure, diastolic blood pressure, heart rate, PR interval, QRS duration and QTcF interval, and concentration time profiles of etamicastat and BIA 5–961 in hypertensive subjects after once-daily administration of placebo or etamicastat (50, 100 and 200 mg/day) for 10 days. Symbols represent mean ± SEM values of 12 and 13 subjects per group.

close to dose-proportionally to the administered dose (Fig. 6), dose proportionality could not be demonstrated using an exponential regression model for C_{max} or $AUC_{0-\infty}$ of both etamicastat and BIA 5–961.

3.2.4. Cardiac evaluation after repeated administration in young and elderly healthy humans

In total, 25 subjects (13 young and 12 elderly) were enrolled. Their demographic and main baseline characteristics are summarized in Table 4. All subjects were Caucasian but 1 Black in the elderly group. One young subject was prematurely discontinued due to a serious AE and was replaced. Twenty-four subjects (12 in each age group) completed the study. Fig. 7 displays the mean change from baseline of supine SBP, DBP, HR, PR interval, QRS duration and QTcF interval in young and elderly healthy subjects after once-daily administrations of etamicastat for 7 days. There was no clinically significant change in supine SBP and DBP in young subjects. However, in elderly subjects, there was approximately 17 mm Hg and 11 mm Hg decreases in SBP and DBP respectively, peaking 6 to 8 h after the last dosing. No clinically significant out-of-range value in vital signs or ECG parameters, HR, PR interval, QRS duration and QTcF interval were observed in young and elderly healthy subjects after once-daily administration of etamicastat for 7 days. In the elderly group, a total of 3 AEs were reported (sciatica, asthenia, and back pain). One serious AE (myopericarditis) occurred in the young group. Paracetamol was administered for the treatment of 2 AEs (sciatica and back pain). All AEs were mild to moderate in intensity but the myopericarditis was severe and led to the subject's discontinuation of the study. Myopericarditis occurred after 2 days of repeated dosing and the subject recovered after 7 days.

The mean plasma concentration-time profiles of etamicastat and BIA 5–961 following the last dose of a 100 mg once-daily regimen of etamicastat for 7 days in elderly and young subjects are displayed in Fig. 7. The corresponding pharmacokinetic parameters are presented in Table 5. The systemic exposure to etamicastat, as assessed by C_{max}, AUC during the dosing interval (AUC_{0-24}), $AUC_{0-\infty}$ and minimum observed concentration (C_{min}), was not significantly different in elderly and young subjects both following repeated doses of etamicastat. Following multiple doses of etamicastat, BIA 5–961 $AUC_{0-\infty}$ and C_{min} were significantly higher in elderly as compared with young subjects.

3.2.5. Cardiac evaluation after repeated administration in hypertensive patients

A total of 23 male volunteers, with ages between 49 and 64 years, were randomized and constituted the safety and pharmacokinetic population. All subjects were Caucasian except one Black. One subject administered 50 mg of etamicastat withdrew on Day 1 due to the occurrence of an AE (ECG repolarization abnormality), and 22 completed the study and constituted the pharmacodynamic population. The demographic and other baseline characteristics of the study population by treatment group are summarized in Table 4. No relevant between-group differences were found. Fig. 8 displays the mean change from baseline of supine SBP, DBP, HR, PR interval, QRS duration and QTcF interval in hypertensive patients subjects after once-daily administrations of etamicastat for 10 days. After 10 days of treatment, the decrease of SBP at day time and night time tended to be more important in subjects who had received 50 mg of etamicastat (−7.6 and −7.4 mm Hg, respectively), 100 mg (−9.1 and −10.2 mm Hg, respectively) and 200 mg (−9.7 and −9.2 mm Hg, respectively) than in subjects who had received placebo (−2.4 and +3.8 mm Hg). After 10 days of treatment, the decrease of DBP at day time and night time also tended to be more marked in subjects who had received 50 mg of etamicastat (−5.0 and −3.6 mm Hg, respectively), 100 mg (−5.2 and −6.8 mm Hg, respectively) and 200 mg (−4.7 and −5.2 mm Hg, respectively) than in subjects who had received placebo (0.0 and +1.2 mm Hg). After 10 days of treatment, no clinically relevant changes of HR at day time and night time were observed in subjects who had received 50 mg of etamicastat (−3.8 and −0.4 bpm, respectively), 100 mg (2.3 and 4.8 bpm, respectively) and 200 mg (+0.3 and −2.5 bpm, respectively) compared to subjects who had received placebo (+0.8 and +0.4 bpm, respectively). No clinically significant out-of-range value in vital signs or ECG parameters, HR, PR interval, QRS duration and QTcF interval were observed in hypertensive subjects after once-daily administrations of etamicastat, for 10 days. No clinically relevant changes were observed in ECG intervals. In particular, no subject had a change from baseline in QTcF of more than 60 ms and there was no significant prolongation of QTcF interval > 480 msec.

Ten patients reported a total of 12 TEAEs: 3 AEs (gamma-glutamyl transferase increase, gout, and diarrhea) in 3 patients with placebo, 1 AE (ECG repolarization abnormality) that led to study discontinuation on Day 1 in a patient with etamicastat 50 mg, 4 AEs (generalized maculopapular rash, pain in a extremity, headache, and vasovagal syncope) in 3 patients with etamicastat 100 mg, and 4 AEs (sciatica, pruritus, eczema, and dry skin) in 3 patients with etamicastat 200 mg. The maculopapular rash was moderate and emerged at Day 10. There were no serious AEs. All AEs were mild (7 cases) or moderate (5 cases) in intensity and recovered without sequelae.

The mean plasma concentration-time profiles of etamicastat and BIA 5–961 following the last dose of a once-daily regimen of etamicastat for 10 days in hypertensive patients are displayed in Fig. 8. The corresponding pharmacokinetic parameters are presented in Table 5. Etamicastat C_{max} was reached 1 h post-dose and declined thereafter with a mean $t_{1/2}$ of 19 to 28 h following repeated administration. Systemic exposure to etamicastat and BIA 5–961 increased less than dose-proportionally with increasing doses.

4. Discussion

Both nepicastat and etamicastat markedly inhibited the activity of native DβH expressed in SK-N-SH human neuroblastoma cells, though the former was more potent than the later with IC_{50}s of 13.3 and 37.2 ng/ml, respectively. On the other hand, the IC_{50} value for reduction of hERG current amplitude by nepicastat (5.6 μg/ml) was 7.9-fold that observed for etamicastat (44.0 μg/ml). Assuming the ratio of IC_{50} values against hERG and DBH as a safety window, then etamicastat (IC_{50} hERG/DβH = 1183) is expected to offer a significant advantage over nepicastat (IC_{50} hERG/DβH = 421). This IC_{50} value of etamicastat (44.0 μg/ml) against hERG is approximately 28 times higher than the plasma concentration (1.6 μg/ml) that was found in healthy volunteers after single administration of 1200 mg, a high well-tolerated dose that produced ca. 80% inhibition of DβB activity [21].

In conscious telemetered *Cynomolgus* monkeys, etamicastat had no substantial effects on arterial blood pressure, HR and the PR interval when administered orally at 15, 45 and 90 mg/kg with the exception of a QT prolongation 14 h following the administration of 45 mg/kg etamicastat, corresponding with the changes observed in HR and RR interval at this time. When the QT interval was corrected for changes in HR/RR interval by calculation of the QTcF interval, this prolongation was still evident; however, when corrected using the individual animal specific correction formula, QTcQ, it was no longer apparent. A slight shortening of the QTcQ interval was noted at 14 h following the administration of 15 mg/kg etamicastat when compared to vehicle, but values remained unchanged when compared to pre-dose baseline. Therefore, this was not considered to be a drug-related effect. No arrhythmia or other changes in the morphology of the electrocardiogram were observed at any dose level of etamicastat. Mean plasma concentrations of etamicastat at 2 and 4 h were 610 and 646, 771 and 1968, and 1055 and 1926 ng/ml at the dose levels of 15, 45 and 90 mg/kg, respectively. Administered orally at 15 and 45 mg/kg/day in female and male *Cynomolgus* monkey for 91 days, etamicastat had no effect on HR and the waveform or intervals of the electrocardiogram. At the highest dose level of 45 mg/kg/day, mean plasma concentrations of etamicastat were 1875 (males) or 2497 (females) and 2895 (males) or 3145 ng/ml (females) on Day 1 and Day 91 of treatment, respectively. Although

male animal species are classically used for safety pharmacology studies, it is not unusual to observe higher plasma concentrations in females than in males. It is also known that females are more susceptible to drug-induced long QT interval and cardiac arrhythmias than males [28,29]. The plasma concentrations measured in monkeys at doses which did not show any deleterious effects, including ECG disturbance, were at least 2 times greater than those measured in humans at the highest cardiovascular active doses (1200 mg) or 10 to 15 times greater than those measured in humans at therapeutic doses (100 to 200 mg). This finding is in agreement with that observed in the hERG study: at 1 μg/ml, no effect was found on the hERG mediated current. Only concentrations 3 to 10 times higher were necessary to affect the delayed rectifier potassium channels.

In the single ascending study in which young healthy volunteers were given once-daily oral doses of placebo or etamicastat from 2 to 1200 mg, the extent of systemic exposure to etamicastat increased in a close but not complete dose-proportional manner. Etamicastat C_{max} occurred quickly after administration, at approximately 1 to 3 h after dosing. Etamicastat elimination appeared to be slower for the highest doses, with elimination half-lives increasing when the dose increased, from 6.6 h at the 20 mg dose to 18.2 h at the 1200 mg dose. There was no clinically significant change in supine SBP, DBP, HR and QTcF interval, though important variability and no clear trend was observed. A total of 18 TEAEs were reported in 13 subjects. There was no serious AE and no AE required the withdrawal of a subject. All AEs were mild to moderate in intensity.

The study that investigated the effect of age on the tolerability and pharmacokinetics of etamicastat in elderly (65 years or older) and young adult (18–45 years) subjects followed a standard design for a phase I study in healthy subjects aiming to investigate the effect of age on the drug pharmacokinetics. This is useful at an early phase of drug development to define the dosage regimes to be tested in elderly subjects enrolled in later therapeutic studies. The cardiovascular safety evaluation comprised changes from baseline of supine SBP, DBP, HR, PR interval, QRS duration and QTcF interval after once-daily administration of etamicastat for 7 days. There was no clinically significant change in supine SBP and DBP in young subjects. However, in elderly subjects, there was approximately 17 mm Hg and 11 mm Hg decreases in supine SBP and DBP, respectively, peaking 6 to 8 h after the last dosing. No clinically significant out-of-range value in vital signs or ECG parameters, HR, PR interval, QRS duration and QTcF interval were observed in young and elderly healthy subjects. The mean plasma concentration-time profiles of etamicastat and BIA 5–961 following the last dose of a 100 mg once-daily regimen of etamicastat for 7 days indicated that the systemic exposure to etamicastat, as assessed by C_{max}, AUC_{0-24}, $AUC_{0-\infty}$ and C_{min}, was not significantly different in elderly and young subjects both following repeated doses of etamicastat. However, the systemic exposure to BIA 5–961, as assessed by $AUC_{0-\infty}$ and C_{min}, were significantly higher in elderly as compared with young subjects.

In hypertensive patients the decrease of SBP at day time and night time tended to be more marked in subjects who received etamicastat (50, 100 and 200 mg) than in subjects who received placebo. After 10 days of treatment, the decrease of DBP at day time and night time also tended to be more marked in subjects who received etamicastat than in subjects who received placebo. This was accompanied by no clinically relevant changes of HR at day time and night time in subjects who received etamicastat (50, 100 and 200 mg) compared to subjects who received placebo. However, it should be underlined that after the last dose of 100 mg etamicastat there was approximately 17 mm Hg decrease in SBP and 11 mm Hg decrease in DBP, peaking 3 to 6 h after the last dosing. No clinically significant out-of-range value in vital signs or ECG parameters, HR, PR interval, QRS duration and QTcF interval were observed in hypertensive subjects after once-daily administrations of etamicastat for 10 days. No clinically relevant changes were observed in ECG intervals. In particular, no subject had a change from baseline in QTcF of more than 60 ms and there was no significant prolongation of QTcF interval >480 msec.

At 100 mg etamicastat, an effective therapeutic dose (ETPC), after repeated administration (trials in elderly/young healthy subjects and in hypertensive patients), C_{max} (0.124, 0.105 and 0.168 μg/ml, respectively) was reached 1 h post-dose and declined, thereafter, with a mean half-life ($t_{1/2}$) of 23 h following the last dose. A 30-fold margin between C_{max} and hERG IC_{50} may suffice for drugs currently undergoing clinical evaluation, but this margin should be increased, particularly for drugs aimed at non-debilitating diseases [30,31]. Binding of [^{14}C]-etamicastat to the human plasma proteins was moderate with a mean of 73.9% (Bial data on file). As such, the ratio between the 44.0 μg/ml IC_{50} for hERG and the 0.035 μg/ml unbound ETPC (44.0/0.044 = 1000) is 33 times higher the 30-fold safety margin. The no-effect concentration of 1.0 μg/ml is 23-fold higher than the unbound clinical C_{max} (0.044 μg/ml). As such, the observed in vitro effect in the hERG assay is highly unlikely to have any clinical impact and this is supported by the review of Redfern et al. [30], which concluded that a greater than 30-fold margin between hERG IC_{50} and unbound clinical C_{max} was sufficiently reassuring.

In conclusion, the blockade of hERG current amplitude by etamicastat together with the QTc interval prolongation observed in conscious telemetered *Cynomolgus* monkeys can be considered as modest with respect to the plasma concentrations found for these effects and to those expected for beneficial cardiovascular activity in humans. These findings and the results of clinical trials in humans suggest that etamicastat is not likely to prolong the QT interval at therapeutic doses.

Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

References

[1] Beliaev A, Learmonth DA, Soares-da-Silva P. Synthesis and biological evaluation of novel, peripherally selective chromanyl imidazolethione-based inhibitors of dopamine beta-hydroxylase. J Med Chem 2006;49:1191–7.

[2] Bonifácio MJ, Igreja B, Wright L, Soares-da-Silva P. Kinetic studies on the inhibition of dopamine-β-hydroxylase by BIA 5–453. PA2 Online 2009;7 [050P (abstract)].

[3] Hegde SS, Friday KF. Dopamine-beta-hydroxylase inhibition: a novel sympathomodulatory approach for the treatment of congestive heart failure. Curr Pharm Des 1998;4:469–79.

[4] Ishii Y, Natsugoe K, Umezawa H. Pharmacological action of FD-008, a new dopamine-beta-hydroxylase inhibitor. Arzneimittelforschung 1975;25:213–5.

[5] Kruse LI, Kaiser C, DeWolf Jr WE, Frazee JS, Ross ST, Wawro J, et al. Multisubstrate inhibitors of dopamine beta-hydroxylase. 2. Structure–activity relationships at the phenethylamine binding site. J Med Chem 1987;30:486–94.

[6] Ohlstein EH, Kruse LI, Ezekiel M, Sherman SS, Erickson R, DeWolf Jr WE, et al. Cardiovascular effects of a new potent dopamine beta-hydroxylase inhibitor in spontaneously hypertensive rats. J Pharmacol Exp Ther 1987;241:554–9.

[7] Stanley WC, Lee K, Johnson LG, Whiting RL, Eglen RM, Hegde SS. Cardiovascular effects of nepicastat (RS-25560-197), a novel dopamine beta-hydroxylase inhibitor. J Cardiovasc Pharmacol 1998;31:963–70.

[8] Sabbah HN, Stanley WC, Sharov VG, Mishima T, Tanimura M, Benedict CR, et al. Effects of dopamine beta-hydroxylase inhibition with nepicastat on the progression of left ventricular dysfunction and remodeling in dogs with chronic heart failure. Circulation 2000;102:1990–5.

[9] Gomes P, Soares-da-Silva P. Dopamine. In: Baden M, editor. Cardiovascular hormone systems: from molecular mechanisms to novel therapeutics. Weinheim: Wiley-VCH; 2008. p. 251–93.

[10] Jose PA, Eisner GM, Felder RA. Role of dopamine receptors in the kidney in the regulation of blood pressure. Curr Opin Nephrol Hypertens 2002;11:87–92.

[11] Jose PA, Soares-da-Silva P, Eisner GM, Felder RA. Dopamine and G protein-coupled receptor kinase 4 in the kidney: role in blood pressure regulation. Biochim Biophys Acta 1802;2010:1259–67.

[12] Soares-da-Silva P. Evidence for a non-precursor dopamine pool in noradrenergic neurones of the dog mesenteric artery. Naunyn Schmiedebergs Arch Pharmacol 1986;333:219–23.

[13] Soares-da-Silva P. A comparison between the pattern of dopamine and noradrenaline release from sympathetic neurones of the dog mesenteric artery. Br J Pharmacol 1987;90:91–8.

[14] Beliaev A, Ferreira H, Learmonth DA, Soares-da-Silva P. Dopamine β-monooxygenase: mechanism, substrates and inhibitors. Curr Enzym Inhib 2009;5:27–43.

[15] Kruse LI, DeWolf Jr WE, Chambers PA, Goodhart PJ. Design and kinetic characterization of multisubstrate inhibitors of dopamine beta-hydroxylase. Biochemistry 1986;25:7271–8.

[16] Igreja B, Loureiro AI, Pires NM, Fernandes-Lopes C, Wright LC, Soares-da-Silva P. Pharmacodynamic and pharmacokinetic profile of dopamine-beta-hydroxylase inhibitor etamicastat in spontaneously hypertensive rats. Hypertension 2011;58:E68 [abstract].
[17] Igreja B, Pires NM, Wright L, Soares-da-Silva P. Effect of combined administration of BIA 5–453 and captopril on blood pressure and heart rate. Hypertension 2008;52: E62 [abstract].
[18] Igreja B, Pires NM, Wright LC, Soares-da-Silva P. Antihypertensive effects of a selective peripheral dopamine beta-hydroxylase inhibitor alone or in combination with other antihypertensive drugs. Hypertension 2011;58:E161–2.
[19] Igreja B, Wright L, Soares-da-Silva P. Long-term lowering of blood pressure levels in the SHR by selective peripheral inhibition of dopamine-b-hydroxylase with BIA 5–453. PA2 Online 2008;6 [087P (abstract)].
[20] Almeida L, Nunes T, Costa R, Rocha JF, Vaz-da-Silva M, Soares-da-Silva P. Etamicastat, a novel dopamine beta-hydroxylase inhibitor: tolerability, pharmacokinetics, and pharmacodynamics in patients with hypertension. Clin Ther 2013;35:1983–96.
[21] Rocha JF, Vaz-da-Silva M, Nunes T, Igreja B, Loureiro AI, Bonifacio MJ, et al. Single-dose tolerability, pharmacokinetics, and pharmacodynamics of etamicastat (BIA 5–453), a new dopamine {beta}-hydroxylase inhibitor, in healthy subjects. J Clin Pharmacol 2012;52:156–70.
[22] Nunes T, Rocha JF, Vaz-da-Silva M, Igreja B, Wright LC, Falcao A, et al. Safety, tolerability, and pharmacokinetics of etamicastat, a novel dopamine-beta-hydroxylase inhibitor, in a rising multiple-dose study in young healthy subjects. Drugs R D 2010; 10:225–42.
[23] Nunes T, Rocha JF, Vaz-da-Silva M, Falcao A, Almeida L, Soares-da-Silva P. Pharmacokinetics and tolerability of etamicastat following single and repeated administration in elderly versus young healthy male subjects: an open-label, single-center, parallel-group study. Clin Ther 2011;33:776–91.
[24] Nagatsu T, Udenfriend S. Photometric assay of dopamine-hydroxylase activity in human blood. Clin Chem 1972;18:980–3.
[25] Verdoorn TA, Draguhn A, Ymer S, Seeburg PH, Sakmann B. Functional properties of recombinant rat GABAA receptors depend upon subunit composition. Neuron 1990;4:919–28.
[26] Loureiro AI, Fernandes-Lopes C, Bonifacio MJ, Wright LC, Soares-da-Silva P. N-acetylation of etamicastat, a reversible dopamine-beta-hydroxylase inhibitor. Drug Metab Dispos 2013;41:2081–6.
[27] Zhou Z, Gong Q, Ye B, Fan Z, Makielski JC, Robertson GA, et al. Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Biophys J 1998;74:230–41.
[28] Lu HR, Remeysen P, Somers K, Saels A, De Clerck F. Female gender is a risk factor for drug-induced long QT and cardiac arrhythmias in an in vivo rabbit model. J Cardiovasc Electrophysiol 2001;12:538–45.
[29] Trepanier-Boulay V, St-Michel C, Tremblay A, Fiset C. Gender-based differences in cardiac repolarization in mouse ventricle. Circ Res 2001;89:437–44.
[30] Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res 2003;58:32–45.
[31] Pollard CE, Valentin JP, Hammond TG. Strategies to reduce the risk of drug-induced QT interval prolongation: a pharmaceutical company perspective. Br J Pharmacol 2008;154:1538–43.