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Research Article

Sustained high blood pressure reduction with etamicastat, a peripheral selective dopamine β-hydroxylase inhibitor

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Abstract

The aim of the present study was to evaluate the influence of chronic inhibition of dopamine ß-hydroxylase by etamicastat on the development of hypertension in the spontaneously hypertensive rat (SHR) and the sustainability of effects on the systolic and diastolic blood pressure in the SHR and the normotensive Wistar–Kyoto rat (WKY). WKY and SHR received etamicastat (10 mg/kg/d) from 5 weeks of age for 35 weeks in drinking water, and cardiovascular assessments were performed on a weekly basis. Etamicastat reduced systolic and diastolic blood pressure when SHRs reached the age of 16 weeks with mean decreases of 37 and 32 mm Hg, respectively, for the subsequent for 24 weeks of treatment, but did not prevent the increase in blood pressure (BP) aged between 5 and 11 week. The BP lowering effect of etamicastat in SHR was reversible on discontinuation and quickly resumed after reinstatement of therapy and was not accompanied by changes in heart rate. Etamicastat affected neither BP nor heart rate in WKY during 36 weeks of treatment. Etamicastat reduced urinary excretion of norepinephrine to a similar extent in WKY and SHR, accompanied by significant increases in urinary dopamine in SHR. Chronic administration of etamicastat did not adversely affected development of animals. Chronic dopamine ß-hydroxylase inhibition with etamicastat effectively decreases BP, although does not prevent the development of hypertension in the SHR. J Am Soc Hypertens 2016;10(3):207–216.

Keywords: Hypertension; dopamine; sympathetic nervous system; norepinephrine.

Introduction

Hypertension is one of the most prevalent conditions worldwide, present in approximately 26.4% of the adult population in 2000 with a projected increase to 29.2% by 2025, affecting 1.5 billion people.[1] Despite availability of several pharmacologic agents from different classes to treat hypertension, approximately 50% of patients do not achieve target blood pressure (BP).[2] Thus, there remains an unmet clinical need for BP control, and this is of particular importance because hypertension constitutes one of the most important cardiovascular risk factors.[3,4]

The sympathetic nervous system (SNS) can alter BP by modulation of cardiac output, peripheral vascular resistance, and renal function. One strategy to decrease sympathetic nerve function is to reduce the biosynthesis of norepinephrine (NE) via inhibition of dopamine β-hydroxylase (DBH; EC 1.14.17.1), the enzyme that catalyzes the conversion of dopamine (DA) to NE in sympathetic nerves.[5] This approach also increases DA levels, which can improve renal function such as renal vasodilation, diuresis, and natriuresis.[6–8]

Several inhibitors of DBH have been reported; however, first- and second-generation examples, such as disulfiram[5] and fusaric acid,[9] were found to be of low potency, with poor selectivity for DBH and having several side effects. One of the most potent DBH inhibitors reported so far is nepicastat.[10] This compound is devoid of some of the

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Conflict of interest: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that B.I., L.C.W., and P.S.d.S. were employees of BIAL–Portela & C[a], S.A. in the previous 3 years.

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problems associated with the first and second generation of DBH inhibitors; however, it was found to cross the blood-brain barrier[10,11] and cause central as well as peripheral effects, which could lead to undesired and potentially dangerous central nervous system adverse effects.

Etamicastat ((R)-5-(2-aminoethyl)-1-(6,8-difluorochroman-3-yl)-1,3-dihydroimidazole-2-thione hydrochloride) is a reversible DBH inhibitor that decreases NE levels in sympathetically innervated tissues and slows SNS drive. Etamicastat was designed to act as a reversible inhibitor of peripheral DBH with limited access to the brain.[11] Our group previously showed single dose etamicastat to dose-dependently decrease BP in SHR and that etamicastat can be combined with others antihypertensive drugs to further decrease BP.[12]

Because antihypertensive treatments are usually chronic, the aim of the present study was to evaluate the effect of chronic DBH inhibition by etamicastat on BP and heart rate (HR), coupled with assessment of catecholamine levels in urine and in different peripheral and brain tissues of SHR and Wistar–Kyoto rat (WKY). SHRs are a well-defined nonclinical model for human essential hypertension and have been widely used for the study and development of novel antihypertensive drugs,[13–17] and WKYs are their normotensive controls.

Materials and Methods

Animals

Male SHR (n = 76) and WKY (n = 76) (Charles River, Barcelona, Spain) were kept with food and water provided ad libitum and maintained under controlled environmental conditions in a colony room (12 hours light/dark cycle, room temperature 22 ± 2°C and humidity 55 ± 15%). Animals were handled for a period of 5 days before the start of noninvasive tail-cuff BP measurements to minimize distress. All experiments were carried out during daylight hours. Tail-cuff BP and HR measurements were made with a Pressure Scanner LE 5700/4 and a Pressure Computer LE 5700 (LETICA Scientific Instruments); animals were kept warmed, and the tail was rinsed with ethanol to improve sensitivity. Experiments were performed according to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes the Portuguese law on animal welfare (Decreto-Lei 113/2013) and conform to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996).

BP and HR Measurements After Acute Etamicastat Administration

SHR and WKY, aged 20–25 weeks, were administered with etamicastat (1, 3, 10, 30, and 100 mg/kg) by oral gavage with at least 7-day interval between treatments (n = 4–8). After gastric load, rats were transferred to a dimly illuminated sound-attenuating room separate from the animal colony room and maintained under identical environmental conditions. BP and HR measurements were performed with a tail cuff, nine hours (t_{max}) after etamicastat administration.

Chronic Administration of Etamicastat

SHR and WKY were continuously administered with etamicastat (10 mg/kg/d) in drinking water for 36 consecutive weeks (ie, aged from 4 to 40 weeks) and BP and HR were measured weekly. Etamicastat dose level was calculated by assessing the body weight and water consumption twice weekly for each animal. During week 28 of treatment (32 weeks old), the administration of etamicastat was stopped and BP and HR were measured on two different days. On the following week, etamicastat was administered in the same regimen as before, starting 3 days before BP and HR measurements. All BP and HR measurements were carried out during light cycle as described above.

Urinary Catecholamines

SHR and WKY (33 weeks old), after chronic dosing of etamicastat (10 mg/Kg/d) for 27 consecutive weeks, were placed in individual metabolic cages for 2 days, and on the second day, urine was collected in tubes containing 1 mL of HCl (6 mol/L) for 24 hours. Urine levels of DA, NE, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were measured. For DA, NE, and DOPAC quantification, samples were first subjected to alumina extraction before injection onto a high-performance liquid chromatography (HPLC) system with electrochemical detection.[18] For HVA determination, the urine samples were filtered (Spin-X 0.22 μm, Costar), diluted 10 times and injected onto a HPLC system with electrochemical detection.[18]

Tissue Catecholamines

At the end of the study, SHR and WKY, which were chronically administered with 10 mg/Kg/d of etamicastat in the drinking water, were killed and heart left ventricle (LV), kidney (K) poles, vas deferens (VD), frontal cortex (FC), parietal cortex (PC), and brain stem (BS) were collected to evaluate NA and DA levels. Tissues were placed overnight at 4°C in tubes containing perchloric acid 0.2 mol/L. Samples were then filtered (Spin-X 0.22 μm, Costar) and injected on a HPLC system with electrochemical detection.

Statistical Analysis

Data are reported as mean ± standard error of the mean of 4–8 animals and analyzed using the Student's *t*-test to compare between vehicle and etamicastat (catecholamines and single point BP and HR measurements) or two-way