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研究課題名（和文）Chronic electrical activation of the arterial baroreflex in hypertensive rats - determining the role of unmyelinated baroreceptors

研究課題名（英文）Chronic electrical activation of the arterial baroreflex in hypertensive rats - determining the role of unmyelinated baroreceptors

研究代表者

ＴＵＲＮＥＲ　ＭＩＣＨＡＥＬ（Turner, Michael）

国立研究開発法人国立循環器病研究センター・研究所・流動研究員

研究者番号：９０７１４２０４

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研究成果の概要（和文）：高血圧の中には薬剤抵抗性のものがあり、新たな治療法の探索が望まれる。その中で電気刺激によって動脈圧受容器反射を活性化し、交感神経活動を抑制して血圧を下げようとするBaroreflex Activation Therapy (BAT)が非薬物治療として注目されている。しかしながら、BATに応答するものと応答しないものがあり、その鑑別が重要である。本研究では高血圧ラットを用いて、圧反射の経路に含まれる無髄線維と有髄線維の違いに着目して降圧メカニズムを探った。

研究成果の概要（英文）：Resistant hypertension is defined as blood pressure that remains above goal in spite of the concurrent use of three different types of antihypertensive agents. Development of new treatment strategies for drug-resistant hypertension is needed. Baroreflex activation therapy (BAT), which stimulates the arterial baroreflex system to reduce sympathetic nerve activity and arterial pressure, is one of non-pharmacological treatmenmts. However, there are responders and non-responders to BAT, and the further understanding of the mechanism of BAT is required to predict the outcome. We explored how BAT decreased arterial pressure from a viewpoint of differences between unmyelinated and myelinated baroreceptor fibers using a rat model of chronic hypertension.

研究分野：　Cardiovascular Physiology

キーワード：　baroreflex　sympathetic system　arterial pressure　hypertension　electrical stimulation　aortic depressor nerve　myelinated fibers　unmyelinated fibers

１．研究開始当初の背景 (Background)

Hypertension is the most common risk factor for life-threatening cardio-vascular diseases. Resistant hypertension is defined as blood pressure (BP) that remains above goal in spite of the concurrent use of three different types of antihypertensive agents. Because commonly used antihypertensive agents are not an effective treatment against resistant hypertension, other treatment strategies such as those utilizing medical engineering may need to be developed. Baroreflex activation therapy (BAT) is a recently revived device-dependent therapy that uses electrical stimulation of baroreceptors, the sensory afferents of the arterial baroreflex, to reduce sympathetic nerve activity and BP. Recent overseas clinical trials using electrical stimulation of the carotid sinus baroreflex have demonstrated effective long-term lowering of BP for more than 3 years in patients with resistant hypertension (Bakris et al. 2012). While the proof of concept for BAT seems to have been established, further investigation is still required before it can be used in standard clinical practice. Especially, there are responders and non-responders to BAT, which strongly indicates the lack of neurophysiological knowledge about how the electrical activation of the baroreflex reduces sympathetic nerve activity and BP.

An often-overlooked issue involved in studies using electrical activation of the baroreflex is the existence of two types of baroreceptor afferents: myelinated (A-fiber) and unmyelinated (C-fiber) (Thoren et al. 1999). Although differences in discharge patterns of these two types of fibers have been well documented, as far as we know, no information is available in the literature as to the differences in the central processing of A-fiber and C-fiber neural pathways during long-term electrical stimulation in normotensive and hypertensive animals. Our proposed project will provide physiological evidence relating to the mechanisms responsible for sustained reduction in BP observed during BAT.

<Reference>

Bakris Gl, Nadim MK, Haller H, Lovett EG, Schafer JE, Bisognano JD. Baroreflex activation therapy provides durable benefit in patients with resistant hypertension: results of long-term follow-up in the Rheos Pivotal Trial. J Am Soc Hypertension. 2012;6:152-158.

Thoren P, Munch PA, Brown AM. Mechanisms for activation of aortic baroreceptor C-fibres in rabbits and rats. Acta Physiol Scand. 1999;166:167-174.

２．研究の目的 (Purpose)

This research will identify the contribution of myelinated (A-fiber) and unmyelinated (C-fiber) type baroreceptor central pathways to the sustained reduction in blood pressure from baroreflex activation therapy (BAT) in rat models of hypertension. A better understanding of how A-fiber and C-fiber baroreceptor central pathways function in hypertension will enable better selection of suitable candidates for this newly revived device based therapy.

３．研究の方法 (Methods)

We used spontaneously hypertensive rats (SHR) as a chronic model of hypertension. As a normotensive control, we used either Sprague-Dawley rats or Wistar-Kyoto (WKY) rats. The animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, which has been approved by the Physiological Society of Japan. The experimental protocols were reviewed and approved by the Animal Subjects Committee at the National Cerebral and Cardiovascular Center.

Animals were anesthetized with an intraperitoneal injection of a mixture of α-chloralose and urethane. A maintenance dose of the anesthetics was given intravenously. Arterial pressure (AP) was measured from a catheter inserted into the femoral vein. Sympathetic nerve activity (SNA) was recorded from a postganglionic branch of the splanchnic sympathetic nerve. The nerve activity signal was amplified with a bandpass filter between 150 and 1000 Hz, then full-wave rectified and low-pass filtered at 30 Hz to quantify SNA.

<u>Protocol 1. Effect of blockade of C-fiber afferents on the open-loop static characteristics of the arterial baroreflex system.</u>

We isolated the right subclavian region of the aortic baroreceptors in Sprague-Dawley rats, and imposed a staircase-wise pressure input between 60 to 180 mmHg, which covered the entire input pressure range of the arterial baroreflex system. The right aortic depressor nerve was identified under a dissecting microscope for later periaxonal

application of resiniferatoxin, which is a potent agonist for transient receptor potential vanilloid-1 (TRPV1) ion channels. Since C-fiber but not A-fiber baroreceptors express TRPV1, application of resiniferatoxin permanently depolarizes the membrane potential, leading to the conduction blockade of C-fiber baroreceptors. Bilateral carotid sinuses, the left aortic depressor nerve, and bilateral vagal nerves were denervated. After recording SNA and AP responses to baroreceptor pressure inputs under control conditions, we applied resiniferatoxin periaxonally so that it disrupted C-fiber baroreceptors alone. Twenty-minutes later, the staircase-wise input protocol was repeated to examine the effects of the C-fiber blockade on the aortic baroreflex function.

Protocol 2. Comparison of dynamic transfer characteristics of A-fiber versus C-fiber baroreceptors in SHR and WKY.

From our previous study (Turner et al. 2014), we found that stimulation of the aortic depressor nerve with high-voltage and low-frequency pulses mainly activated C-fiber baroreceptors whereas stimulation with low-voltage and high-frequency pulses mainly activated A-fiber baroreceptors in Sprague-Dawley rats. By combining these stimulation settings with a binary white noise input, we separately identified dynamic characteristics of the A-fiber baroreceptors and C-fiber baroreceptors. The former exhibited significant derivative characteristics, whereas the latter showed weak derivative characteristics. These results indicate that A-fiber baroreceptors contribute to rapid AP regulation, whereas C-fiber baroreceptors contribute to more sustained AP regulation.

Based on the above knowledge, we compared dynamic transfer characteristics of A-fiber versus C-fiber baroreceptors in SHR and WKY. Bilateral vagal nerves, carotid sinus nerves and aortic depressor nerves were sectioned. The sectioned central end of the left aortic depressor nerve was stimulated by the two types of binary white noise inputs. We calculated transfer function from aortic depressor nerve stimulation to SNA (the central arc), that from SNA to AP (the peripheral arc), and that from nerve stimulation to AP (Stim-AP arc).

<Reference>

Turner MJ, Kawada T, Shimizu S, Sugimachi M. Sustained reduction in blood pressure from electrical activation of the baroreflex is mediated by the central pathway of unmyelinated baroreceptors. Life Sci. 2014;106:40-49.

Protocol 3. Effects of baroreflex activation therapy (BAT) on the arterial baroreflex system.

Baroreflex activation therapy uses stimulation of unilateral or bilateral carotid sinus baroreceptor stimulation to reduce AP. As the current BAT system does not monitor dynamic changes in AP, it could impede native AP regulation by the other unstimulated baroreceptors including the aortic baroreceptors. To examine whether BAT impede the native AP regulation, we isolated bilateral carotid sinuses and imposed Gaussian white noise to identify the dynamic characteristics of the carotid sinus baroreflex with or without electrical stimulation of the aortic depressor nerve. In this context, the aortic depressor nerve is used as a target of BAT, and the carotid sinus baroreflexes are supposed to be unstimulated native baroreflexes. The protocol was performed on both normotensive WKY rats and SHR.

4. 研究成果 (Results)

Result 1. Effect of blockade of C-fiber afferents on the open-loop static characteristics of the arterial baroreflex system.

The input-output relationship between baroreceptor input pressure and efferent AP revealed an inverse sigmoid curve. Periaxonal application of resiniferatoxin resulted in a lower response range and higher minimum AP, but it did not affect the maximum slope of the sigmoidal relationship. These results suggest that A-fiber baroreceptors can regulate AP when systemic AP is around the normal operating range. In contrast, C-fiber baroreceptors are critically important for AP reduction when systemic AP is raised above the normal operating range (list of published papers #3).

Result 2. Comparison of dynamic transfer characteristics of A-fiber versus C-fiber baroreceptors in SHR and WKY..

In WKY, the central arc transfer function relating to the A-fiber baroreceptors showed strong derivative characteristics, whereas that relating to the C-fiber baroreceptors exhibited non-derivative characteristics. In SHR, the derivative characteristics are pronounced for the A-fiber baroreceptors. The derivative characteristics are also

pronounced for C-fiber baroreceptors but with a reduction of dynamic gain in the lower frequency range. Hence, the dynamic characteristics of the A-fiber central pathway are enhanced in the high frequency range, and those of the C-fiber central pathway are attenuated in the low frequency range. In other words, differences in the dynamic baroreflex characteristics of unmyelinated and myelinated central pathways are less evident in SHR, which may account for the inability of SHR to reduce long-term AP via C-fiber baroreceptors (list of published papers #2).

Protocol 3. Effects of baroreflex activation therapy (BAT) on the arterial baroreflex system.

The dynamic characteristics of baroreflex neural arc from pressure inputs to carotid sinus baroreceptors to efferent SNA revealed derivative characteristics. These characteristics were hardly affected by application of BAT through the aortic depressor nerve in WKY or SHR. Hence BAT is unlikely to impede the dynamic AP regulation afforded by the unstimulated native arterial baroreflexes.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

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〔図書〕（計 0 件）
なし

〔産業財産権〕
○出願状況（計 0 件）
なし

○取得状況（計 0 件）
なし

〔その他〕
なし

６．研究組織
(1)研究代表者
ターナー マイケル（TURNER, Michael）
国立循環器病研究センター・
研究所・流動研究員
研究者番号：90714204

(2)研究分担者
なし

(3)連携研究者
なし