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Review

Rat aorta as a pharmacological tool for *in vitro* and *in vivo* studies

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ABSTRACT

Rat aorta assay provides a low cost and rapid platform, especially for preclinical in vivo models. The signaling pathways of the analog on the vessels could be evaluated separately on the endothelium or smooth muscle cells by rings of the rat aorta in vitro. The rat aorta is used for angiogenesis modeling to integrate the benefits of the both in vivo and in vitro models. These explain the importance and usage of rat aorta in researches. Furthermore, about 4503 articles have been published with the key word "rat aorta" in title or abstract from 1955 until the end of 2013 in Medline. In this review, these articles were organized into two main categories: in vivo and in vitro studies. The in vitro section focused on the rat aorta model, as a tool for evaluate the mechanism of vasodilation, vasoconstriction and angiogenesis. In the in vivo section, the most important usage of this tissue was evaluated. Also, the vasotonic signaling pathways in the vessel are explained briefly and some rat aorta applications in vitro and in vivo have been discussed.

Contents

Abbreviations: BH_4, tetrahydrobiopterin; cGMP, cyclic guanine monophosphate; COX, cyclooxygenase; cNOS, constitutive NOS; DAG, diacylglycerol; DMEM, Dulbecco's modified eagle's medium; eNOS, endothelial NOS; EDHF, endothelium dependent hyperpolarization factor; EGTA, ethylene glycol tetra acetic acid; ET_B, endothelin-B; ET_A, endothelin-A; FBS, fetal bovine serum; 5HT, serotonin; iNOS, inducible NOS; IP_3R, inositol 1,4,5-triphosphate receptor; IP_3, inositol 1,4,5-triphosphate; IHC, immunohistochemistry; K_{IR}, inward rectifier K^+; MAP kinase, mitogen-activated protein kinase; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal NOS; PG, prostaglandin; PI_3-kinase, phosphoinositide 3-kinase; PKG, protein kinase G; PKC, protein kinase C; ROCC, receptor-operated Ca^{2+} channel; RyRs, ryanodine receptors; ROS, reactive oxygen species; RT-PCR, reverse transcription polymerase chain reaction; RASMC, rat aortic smooth muscle cells; sG, soluble guanylyl cyclase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATP_{ase}; SK_{Ca}, small-conductance calcium activated potassium channel; SR, sarcoplasmic reticulum; TXA_2, thromboxane A_2; tNOS, total NOS; VSMC, vascular smooth muscle cell; VOCC, voltage-operated Ca^{2+} channel; VP, vasopressin; VC, vascular calcification.

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1. Introduction

The vascular system has always been a major field of interest to medical researchers [1,2]. Availability of appropriate non-cadaveric specimens, may however be quite difficult [3].

According to available literature, using rat aorta, as a substitute for human vascular tissue, and also as a competent model that bridges the gap between in vivo and in vitro model studies [4], dates back to the early 1960s [5–7]. Respiration of blood vessels [8], and experimental models of hypertension [9], atherosclerosis [10], metabolic pathways [11,12] and aortic aneurysms [13], are some examples of major research fields done in the era of introducing rat aortic model.

The adaptation of different species and vessel types [14–16], as well as its advantage over similar models that employ cultured tissues and cells [17], further enhanced the status and rank of using rat aortic model among researchers. It should however be noted that recent improvements in this field did not reduce the importance of this model, but also increased its reputation as a suitable model among researchers in diverse fields of vascular system-associated conditions. Scrutinizing the process of angiogenesis [18] and its contributions [19,20] using state-of-the-art 3-D models of rat aorta [21], searching for cutting-edge vascular imaging techniques [22], investigating new drug delivery systems [23], evaluating vascular prostheses [24], and looking closely into vascular aging [25–28] and atherosclerosis [29,30], are some instances of modern use of rat aortic model. Among these research fields, vasoconstriction [31–39] and vasorelaxation [40–60] have come under particular focus by scientists. This study aims to discuss the role of rat aortic model in investigating vasoconstriction and relaxation, with emphasis on and their underlying mechanisms.

2. In vitro studies

2.1. Dissection and preparation of rat aorta

To obtain the thoracic aorta, adult rats (Table 1) are sacrificed and dissected to harvest the aorta after an appropriate anesthesia. The obtained tissue is cleaned from surrounding excess fat and connective tissue, and cut into rings which are placed in organ bath solutions usually maintained at 37 °C with a pH of 7.4 for isometric force recording. Two identical platinum or stainless steel hooks are introduced through the lumen of the aortic rings, with one fixed in the bath chamber while the other is attached to a force transducer connected to an analyzing and recording machine.

The rings are equilibrated at a fixed resting tension for a definite period. In the interim, the bathing solution is refreshed at particular intervals (Table 1) [61–68]. To reach an optimal tension, the contraction is induced by using KCl solution (45 mM). Maximal contraction is evoked at the optimal tension [69].

If an endothelium-denuded ring is required, its internal surface is gently rubbed [70] with a cotton stick moistened with physiological salt solution [61,71], 18 gauge needle [72], metal or stainless steel rod [67,73], or a pair of watchmaker's forceps [65,69,74–76]. The rings are then immersed in organ baths containing buffer solutions with various ingredients in different experiments (Table 2).

2.2. Determination of viability, maximum tissue contractility and integrity of the endothelium

A contractive response to KCl confirms the viability of the tissue. Maximum tissue contractility is assessed in the same way. To establish the integrity of the endothelium, the relaxation response to muscarinic agonist such as acetylcholine in pre-contracted rings with phenylephrine is tested. Rings with functional endothelium show significant relaxation [61–63,69,74,77–82] (Table 3).

2.3. Prepared tissue usage

Prepared rat aortic rings have been used to evaluate the effect of various natural or synthetic compounds and drugs on vascular tension namely diazoxide [83], aminophylline [84], caffeine [85], halothane and isoflurane [71,86], chloroethylclonidine [81], Iso-S-petasin [87], synthetic calciseptine and FS2 (a snake venom peptide homologous) [88], Jermok [89], Dihydropyridineethylester [90], simvastatin [82], antidepressants [91], coenzyme Q10 [92], sodium nitroprusside [93], dimethyloxalylglycine [94], isosteviol [40], betaine [27], cocaine [95], inhibitors of SIRT1 (nicotinamide, sirtinol, EX527) [26], zinc [29], angiotensin II [96], flavonoids [47,97–99], estrogens [100–111] other miscellaneous herbal ingredients [75,80,105,112–121], and malaria parasite [122].