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EFFECTS OF GLUTATHIONE ON RELAXATION OF THE ISOLATED RAT AORTA

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ณัฐธยาน์ เชาว์ธนาพัฒน์: ฤทธิ์ของกลูตาไทโอนต่อการคลายตัวของหลอดเลือดแดงใหญ่ที่แยกออกจากกายหนูขาว. (EFFECTS OF GLUTATHIONE ON RELAXATION OF THE ISOLATED RAT AORTA) อ. ที่ปรึกษา : ผศ. ดร. สุรีย์ เจียรณ์มงคล, อ. ที่ปรึกษาร่วม : รศ.ดร. ประสาน ธรรมอุปกรณ์, 109 หน้า.

จากรายงานเกี่ยวกับภาวะที่มีการลดลงของกลูตาไทโอนทำให้เกิดผลต่อการทำงานหลอดเลือด ดังนั้นงานวิจัยนี้จึงทำการศึกษากลไกการออกฤทธิ์ของกลูตาไทโอนในหลอดเลือดแดงใหญ่ที่แยกออกจากกายหนูขาวส่วนทรวงอก ซึ่งผลการทดลองพบว่า กลูตาไทโอนสามารถทำให้เกิดการคลายตัวของหลอดเลือดที่ได้รับ PE โดยการคลายตัวนั้นเกิดขึ้นทั้งในแบบอาศัยและไม่อาศัยเยื่อบุผนังหลอดเลือด อย่างไรก็ตามอิทธิพลของเยื่อบุผนังหลอดเลือดทำให้ผลของกลูตาไทโอนที่ทำให้เกิดการคลายตัวมีความแตกต่างกันในเรื่องของลักษณะของ tracing และระดับความตึงตัวที่ลดลง นอกจากนี้ยังพบว่าการคลายตัวของหลอดเลือดแบบอาศัยเยื่อบุผนังหลอดเลือดสามารถถูกยับยั้งได้ด้วย L-NAME, methylene blue และ glibenclamide ส่วนการคลายตัวแบบไม่อาศัยเยื่อบุผนังหลอดเลือดถูกยับยั้งได้ด้วย glibenclamide เท่านั้น ดังนั้นจึงอาจสรุปได้ว่ากลูตาไทโอนกระตุ้นการคลายตัวหลอดเลือดได้โดยการกระตุ้นผ่านทางกลไกของ NO-cGMP และ hyperpolarizing pathway นอกจากนี้จากการศึกษายังแสดงให้เห็นว่าแคลเซียมจากภายนอกเซลล์มีอิทธิพลต่อการคลายตัวของเนื้อเยื่อหลอดเลือด โดยที่ผลของกลูตาไทโอนสามารถถูกยับยั้งได้ด้วย EGTA แต่ไม่สามารถยับยั้งด้วย BAPTA-AM ยิ่งไปกว่านั้นกลูตาไทโอน ยังออกฤทธิ์เสริมฤทธิ์ของ Ach ในการคลายตัวของหลอดเลือด โดยไม่มีผลต่อการออกฤทธิ์ของ SNP แต่อย่างใด ทำให้สรุปได้ว่า กลูตาไทโอนมีผลทำให้หลอดเลือดคลายตัวโดยกระตุ้นการสร้าง NO ได้จากการเพิ่มการเคลื่อนที่เข้าของแคลเซียมจากภายนอกเข้าสู่ภายในเซลล์ของเยื่อบุผนังหลอดเลือด โดยไม่มีผลต่อการสร้าง cGMP ที่กล้ามเนื้อเรียบหลอดเลือด กลูตาไทโอนยังมีผลโดยตรงต่อการยับยั้งการหดตัวของเซลล์กล้ามเนื้อเรียบหลอดเลือดได้ในสภาวะต่างๆ ซึ่งจากผลการทดลองแสดงให้เห็นว่า กลูตาไทโอนออกฤทธิ์ยับยั้งการหดตัวของหลอดเลือดที่ได้รับการกระตุ้นให้หดตัวด้วย PE, 5-HT และ histamine แต่ไม่มีผลต่อการหดตัวของกล้ามเนื้อจากการกระตุ้นด้วย KCl, TEA และ PMA นอกจากนี้ กลูตาไทโอนสามารถลดการเคลื่อนที่เข้าของแคลเซียมจากภายนอกเข้าสู่ภายในเซลล์ในสภาวะที่เป็น high K^+ depolarization ได้ ดังนั้นจึงอาจสรุปในภาพรวมได้ว่า กลูตาไทโอนมีผลต่อการควบคุมความตึงตัวของหลอดเลือดโดยการรบกวนการเคลื่อนที่เข้าของแคลเซียมที่ Ca^{2+} channel บนผนังเยื่อหุ้มเซลล์ ตลอดจนรบกวนการปลดปล่อยของแคลเซียมจากภายในเซลล์ ซึ่งสัมพันธ์กับ IP_3 signaling จากผลทั้งหมดนี้กลูตาไทโอนสามารถกระตุ้นกลไก NO-cGMP ของเซลล์เยื่อบุผนังหลอดเลือด และยังมีผลในการลดการเคลื่อนที่เข้าของแคลเซียมจากภายนอกเข้าสู่ภายในเซลล์ รวมทั้งมีผลในการลดการปลดปล่อยของแคลเซียมจากภายในเซลล์ของกล้ามเนื้อเรียบหลอดเลือดได้

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NATTAYA CHAOTHANAPHAT : EFFECTS OF GLUTATHIONE ON RELAXATION OF THE ISOLATED RAT AORTA.
THESIS ADVISOR: ASST.PROF. SUREE JIANMONGKOL, Ph.D., THESIS COADVISOR: ASSOC.PROF. PRASAN DHUMMA-UPAKORN, Ph.D., 109 pp.

It has been reported that depletion of GSH affected the function of vascular system, including control of vascular tone. This study investigated the direct effects of GSH on vascular tension, using the *in vitro* model of isolated rat thoracic aorta. The results showed that GSH significantly induced both endothelium-dependent and endothelium-independent relaxation of aortic preparations which were precontracted with PE. However, the presence of endothelium influenced the response of aortic muscles toward treatment of GSH, resulting in the difference of the characteristic of the tracing profiles and degree of relaxation. The endothelium-dependent relaxation were abolished by pretreatment with L-NAME, methylene blue and glibenclamide whereas the endothelium-independent relaxation were reduced only by pretreatment with glibenclamide. Therefore, the mechanisms of GSH-induced relaxation involved the NO-cGMP pathway as well as membrane hyperpolarization pathway. Moreover, extracellular Ca^{2+} could also determine the effects of GSH on vasorelaxation because the presence of EGTA, but not BAPTA-AM, could interfere the vasorelaxation. In addition, GSH was able to enhance the effects of Ach, but not SNP on vasorelaxation. Hence, it was likely that GSH enhanced the production of NO via increasing Ca^{2+} influx in endothelium cell, but had no effect on the production of cGMP in vascular smooth muscle cell. Furthermore, GSH was able to directly inhibit the contraction of smooth muscle in certain conditions. The results showed that GSH could elicit its inhibitory action toward the contraction induced by PE, 5-HT and histamine but not those induced by KCl, TEA and PMA. In addition, GSH also inhibited the Ca^{2+} influx in high K^+-depolarizing solution. Thus, these findings suggested that GSH modulated the vascular tone via interference of Ca^{2+} influx through membrane Ca^{2+} channels on vascular smooth cells. Moreover, GSH could partially interfere Ca^{2+}-release from internal storage which coupled to IP_3 signaling. Taken together, GSH could directly modulate the control of vascular tone by enhance the NO-cGMP pathway in endothelium cells as well as disrupt the Ca^{2+} influx and Ca^{2+}-release in the smooth muscle cells.

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LIST OF ABBREVIATIONS

AC	adenylate cyclase
ACh	acetylcholine
ANOVA	one-way analysis of variance
ATP	adenosine 5'- triphosohate
ATR	atropine
Ca^{2+}	calcium ion
$[Ca^{2+}]_I$	intracellular calcium
cAMP	cyclic adenosine 3', 5'- monophosphate
cGMP	cyclic guanosine 3',5'- monophosphate
COX	cyclooxygenase
DAG	diacylglycerol
EDHF	endothelium-derived hyperpolarizing factor
EDRF	endothelium-derived relaxing factors
sGC	soluble guanylate cyclase
GLIBEN	glibenclamide
GSH	glutathione
GTP	guanosine 5`- triphosohate
5-HT	serotonin
Ibu	Ibuprofen
IP_3	inositol 1, 4, 5- trisphosphate
IRT	increase resting tone
K^+	potassium ion
KCl	potassium chioride
KHS	Krebs-Henseleit solution
L-NAME	N^G-nitro-L-arginine methyl ester
M	molar
MB	methylene blue
ml	milliltre
MLCK	myosin light chain kinase

mM	milimolar
NAC	N-acetyl-cysteine
NO	nitric oxide
SR	sarcoplasmic reticulum
SHR	spontaneous hypertensive rat
PE	phenylephrine
PLC	phospholipase C
PMA	phorbal-12-myristate-13-acetate
PP	propranolol
ROC	receptor-operated calcium channels
S.E.M.	standard error of mean
SNP	sodium nitropusside
SOC	store- operated calcium channels
TEA	tetraethylammonium chloride
μM	micromolar
μg	microgram
VOC	voltage-operated calcium channels
VSM	vascular smooth muscle