

Basal Tone of Blood Vessel: Cyclic Guanosine Monophosphate (cGMP) Dependent or Not? An Experiment with Soluble Guanylyl Cyclase Inhibitor

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ABSTRACT

Background: Nitric oxide (NO) is a signaling molecule that is produced constitutively in endothelium. It maintains the basal tone of blood vessel. It can produce vasodilation through cyclic guanosine monophosphate (cGMP) dependent and independent mechanisms. The aim of our study was to identify whether the basal tone of blood vessel is maintained by cGMP dependent mechanism or not. For this we have used soluble guanylyl cyclase inhibitor, 1H-[1,2,4] oxidiazolo [4,3-a]quinoxalin-1-one (ODQ), which will prevent the formation of cGMP. **Methods:** Wistar rat isolated hind limb preparation was used in our study. Rat was anaesthetized and the upper half of the body was removed and the abdominal aorta was cannulated. It was perfused with physiological salt solution and the blood pressure was recorded using a pressure transducer connected to CMC data acquisition system (CMCdaq). The rate of perfusion was fixed by using peristaltic pump at 4ml/min. The rat was perfused with ODQ for 10 minutes and was again perfused with extracellular fluid. **Results:** ODQ did not produce any significant change in the mean arterial pressure. This shows that cGMP is not required for the basal tone of blood vessel as there was no change in the mean pressure even after blocking cGMP production by ODQ. **Conclusion:** We concluded that basal tone of blood vessel maintained by NO is cGMP independent.

Keywords: Vascular tone, cGMP, ODQ, Nitric oxide, soluble Guanylyl cyclase.

INTRODUCTION

Nitric oxide (NO) is a vasodilator which is produced from L- arginine by nitric oxide synthase enzyme (NOS). Of the three isoforms of NOS namely iNOS, eNOS and nNOS, eNOS is present in the endothelium which is constitutively producing nitric oxide.^[1] Nitric oxide can produce vasodilatation through cyclic guanosine monophosphate (cGMP) dependent and independent pathways.^[2] In cGMP dependent pathway, NO activates sGC which will convert GTP to cGMP. cGMP activates Protein kinase G and produces vasodilatation through activation of myosin light chain phosphatase. cGMP independent vasodilatation is either through activation of SERCA or stimulation of calcium dependent potassium channel.^[3] NO signaling pathway is driving the search of treatment for hypertension, endothelial dysfunction, cardiac failure, atherogenesis and Raynaud's disease.^[4]

Here we aimed to see whether the base line tension due to nitric oxide is cGMP dependent or not. Previous studies had used methylene blue or

LY83583 as sGC inhibitors, but they are not specific as they can inhibit NO synthase as well.^[5] To overcome this, we have used a specific sGC inhibitor 1H-[1,2,4] oxidiazolo [4,3-a]quinoxalin-1-one (ODQ), in the presence of which cGMP will not be produced. Grathwaite et al reported that ODQ can inhibit sGC in a noncompetitive manner and it would not inactivate NO or would not inhibit sGC independent actions of NO.^[6] ODQ was found to decrease the relaxation by NO but did not prevent the complete relaxation pointing that sGC independent mechanisms are also present in vasodilatation.^[5,7]

MATERIALS AND METHODS

Male Wistar rats weighing between 270 – 300 grams were used in the study. Rats were administered ketamine at a dose of 100 mg/kg body weight and xylazine at a dose of 1.87 mg/kg body weight intraperitoneally to anaesthetize them. Rats were restrained on a dissection board. Limbs were fixed with adhesive. A vertical incision was made from xiphisternum up to pubic area to expose abdominal cavity. Skin and muscle layers were incised and retracted laterally. For the better visualization of vessel, bowel was taken out. Abdominal aorta was identified above the lumbar region of vertebra. Connective tissue and fat around the vessel were removed to access it.

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Cannulation was done in the abdominal aorta distal to renal artery and proximal to iliac artery bifurcation. A spatula was kept under this region of aorta. A ligature was applied on the proximal part of aorta. After the ligation, trunk of the rat was transected proximal to ligatures using bone cutter and the upper half of the body was removed. The abdominal aorta was cannulated using 24G cannula which was flushed with heparin. The distal end of aorta was ligated with the cannula to fix it in the place. Inferior vena cava was opened for the effluent to flush out. Subsequently the cannula was connected to the tube from peristaltic pump. The line was also connected to pressure transducer and CMCdaq which would record the pressure on a computer.

Modified Langendehoff method was used to perfuse the hind limb. Two or three reservoirs were used. The perfusate was kept at 37 ° C and was gassed with carbogen (95% oxygen + 5% carbon dioxide as bicarbonate is present in the extracellular fluid). The composition of the solution was as follows (in mmol/L): NaCl 100; KCl 3; CaCl₂ 1.3; MgCl₂ 2; Na₂HPO₄ 2; NaH₂PO₄ 0.5; NaHCO₃ 25; HEPES 10; Glucose 5, pH 7.4 with 1 molar NaOH. Isolated hind limb preparation was perfused with extracellular fluid until it stabilized. The rate of the perfusion pump was fixed at 4ml/min. The rat was perfused with ODQ for 10 minutes and was again perfused with extracellular fluid. As we know that mean arterial pressure depends on cardiac output and total peripheral resistance, the change in pressure after perfusion of ODQ depends on the vessel tone change, as the stroke volume remains constant.

All salts in the solution were purchased from SIGMA. ODQ was also purchased from SIGMA. ODQ was dissolved in dimethyl sulphoxide (DMSO).

Statistical Analysis

Statistical analysis was done using Wilcoxon signed rank (WSR) test to compare pressures before and after ODQ. (SPSS version 20)

RESULTS

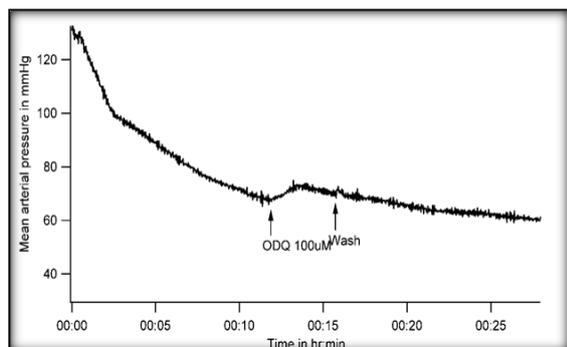


Figure 1: Representative data showing the change in mean arterial pressure in the presence of ODQ.

ODQ (100µM), the sGC inhibitor, did not produce any significant change in mean pressure. The mean pressure changed from 71 to 78 mmHg in the presence of ODQ (n = 6, P = 0.109 with WSR test). Representative data showing the change in mean arterial pressure in the presence of ODQ is given [Figure 1].

DISCUSSION

Nitric oxide, also called as endothelium derived relaxing factor (EDRF), is produced in the endothelium by eNOS. Nitric oxide maintains the basal tone of vessel. It can produce vasodilation in cGMP dependent and independent pathway.^[3] Nitric oxide activates sGC which converts GTP to cGMP. ODQ inhibits sGC and prevents the formation of cGMP.^[6] In our study pressure change with ODQ was recorded to see whether basal tone of blood vessel is cGMP dependent or independent. ODQ did not produce any significant change in the mean arterial pressure. This shows that basal tone of blood vessel is maintained by nitric oxide in a cGMP independent mechanism. So when NO is used as a treatment option, we have to consider that the basal tone of blood vessel is cGMP independent. The drugs that are specifically using the cGMP pathway may not be useful for maintaining the basal tone of blood vessel.

CONCLUSION

It may be concluded that basal tone of blood vessel maintained by NO is cGMP independent as sGC inhibitor did not produce any change in basal pressure. If confirmed with further experiments, this will significantly influence future research in management of various conditions like hypertension and atherosclerosis.

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