ORIGINAL ARTICLE

Evaluation of the Vasoplegic impact of Papaverine in the rat aorta

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Abstract

Objective: To identify the degree of vasoplegic affinity of papaverine to rat thoracic aortas following constriction caused by adrenalin, serotonin and potassium chloride in an in-vitro model.

Methods: The in vitro vasoplegic efficacy of papaverine against adrenalin (10^{-5} M), serotonin (5HT) (10^{-4} M), and KCl (60 mM) was assessed, using a rat aortic vasospasm model in an organ bath. First, aortic rings were constricted with a submaximal dose of vasoconstrictor agents. The samples were then incubated with papaverine ($3x10^{-4}$ M) for 20 minutes, followed by readministration of the same vasoconstrictor agents. The first vasospastic response (before papaverine incubation) and the new vasoconstrictor responses (after papaverine incubation) of the vessels were then compared.

Results: The vasoplegic effect of vasoconstrictor agents in decreasing order was observed as adrenalin>KCl>5HT. This different affinity for the vasoplegic effect is considered to be a temporary impact of the drugs and the maximal inhibition of vasoconstriction was detected for the adrenalin receptor.

Conclusion: The relevance of the macromolecules is responsible for the permanent efficacy of the drugs. Different degrees of vasoconstriction were also obtained after papaverine administration, which suggests that different responses can occur as a result of different stimulation of receptor modulators.

Keywords: Papaverin, Vasoplegic effect, Vasoconstrictor agents. (JPMA 64: 660; 2014)

Introduction

Vasospasm is an important determinant for systemic vascular events such as ischaemic heart disease and cerebral haemorrhage.[1,2] For this reason, many studies have focused on finding a solution to this problem. Various therapeutic agents have been tested in different studies as candidates for developing new treatment strategies.[1] One of these agents, papaverine, is a potent vasoplegic substance that blocks the breakdown of cyclic nucleotides and can also cause vasodilatation by inhibiting phosphodiesterase.[3] The vasodilator effects of papaverine have been recognized for many years[4] and its receptor affinities have also been investigated.[4]

Papaverine has been used for a number of different vasospastic and vascular disorders, but its efficacy appears to vary under different situations.[5] The possible therapeutic efficacy of its vasodilator effect has been investigated in many studies on wound healing and aneurysm surgery.[5,6] However, the affinity of papaverine for different vasoconstrictor substances has not yet been clarified, so the interaction between the vasospastic agents and papaverine remains unknown.

The aim of this study was to identify the degree of vasoplegic affinity of papaverine to rat thoracic aortas following constriction with the vasoconstrictor agents adrenalin, serotonin (5HT) and KCl, using an in vitro organ bath model established for the study.

Material and Methods

Approval was obtained from the Ethics in Local Animal Experimentation Committee. This study was carried out at the Department of Cardiovascular Surgery and Pharmacology, Faculty of Medicine, Dicle University, Diyarbakir, Turkey between February 2013 and June 2013. Two mature male Wistar rats weighing 280-300 g were used for the study.

The rats were sacrificed by cervical dislocation. The chest cavities were opened and the thoracic aortas were excised. Excised aortas were placed into Petri dishes containing Krebs solution (NaCl, 119mM; KCl, 4.7mM; MgSO4, 1.5mM; KH2PO4, 1.2mM; CaCl2, 2.5mM; NaHCO3, 25mM; glucose, 11mM; pH 7.40±0.05). The surrounding fatty and connective tissues were removed, taking care to prevent possible

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damage to the endothelial tissue. The aortas were then divided into pieces and 3-4mm vascular rings were obtained for each sample group. The rings were placed one by one into 4 layered organ baths (LE 01.046, Panlab, S.L. Barcelona, Spain) containing Krebs solution and fixed with a tight rope to the transducer (Letica TRI 201, Panlab,. S.L., Barcelona, Spain). The temperature of the organ bath was adjusted to 37°C. The Krebs solutions were freshened every 15 minutes. The organ bath was aerated with 95% O_2 - 5% CO_2 gas during the test.

Aortic rings were allowed to rest for one hour at below the 1.5g resting tension after placement in the organ bath. The different vasoconstrictor agents were then administered to each separated ring. One of the rings was contracted with adrenalin (10^{-5} M), one with 5HT (10^{-4} M) and one with KCl (60mM). After each contraction, the aortic rings were washed several times with Krebs solution and then allowed to rest for 30 minutes. Afterwards, the rings were incubated with papaverine ($3x10^{-4}$ M) for 20 minutes. After this incubation, the baths were washed several times and the vessel rings were again left to rest for 30 minutes in Krebs solution. Finally, the rings were again contracted with adrenalin (10^{-5} M), 5HT (10^{-4}M) or KCl (60mM) (Figure-1). (Each test was repeated twice, for confirmation.)

The contractile responses of the vessels following chemical administration were recorded into a computer system via an isometric transducer kit and the obtained response curves were interpreted.

Statistical Analyse

The responses of groups were compared with independent t test. All statistical procedures were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL). A p value of 0.05 was considered statistically significant.

Results

Adrenalin was the most effective vasoconstrictor agent in terms of showing the vasoplegic effect of papaverine. The magnitude of the vasoplegic effect of vasoconstrictor agents, in decreasing order, was Adrenaline >KCl >5HT, as shown in Figure-2-A and 2-B. The response of Adrenaline was statistically significant ($p<0.002$) than others. Although, marked decrement was obtained with KCl than 5 HT, both two agents have

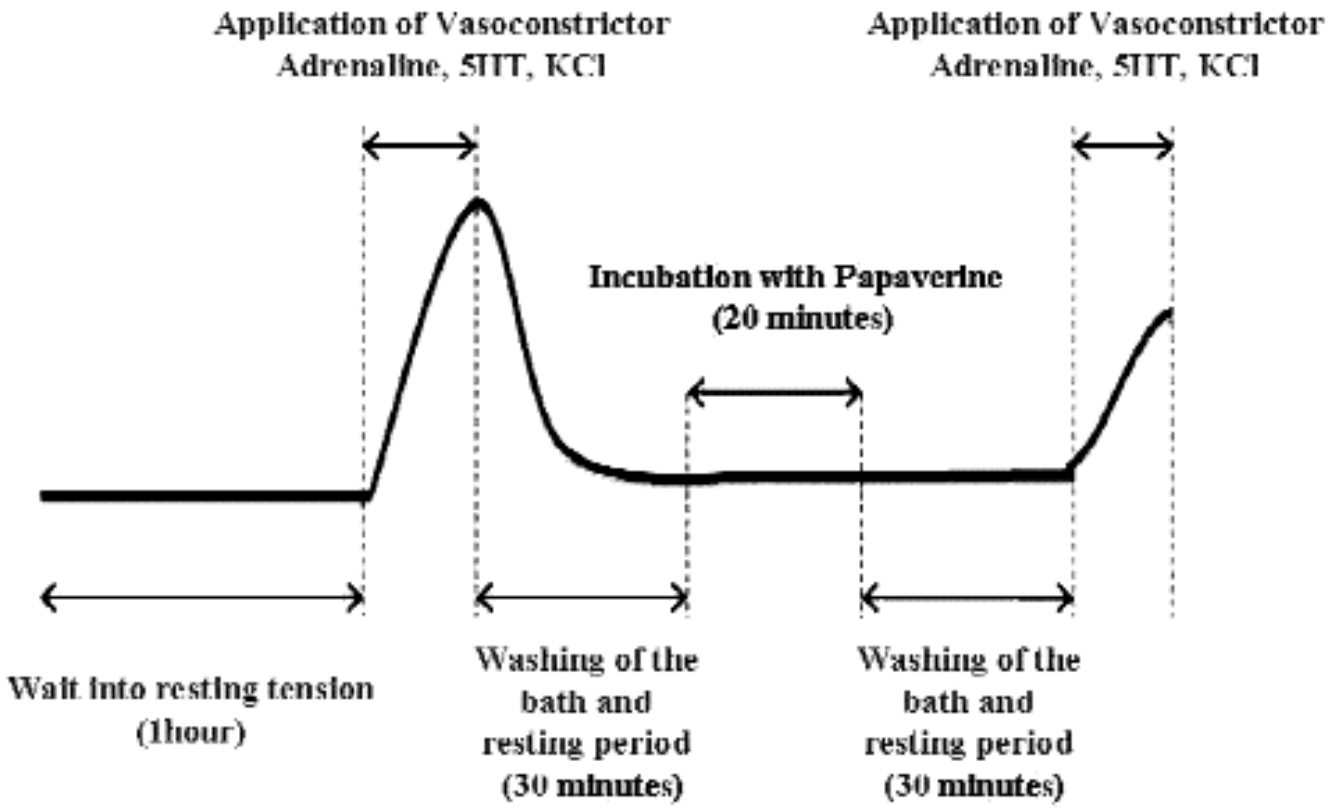

Figure-1: The study stages protocol in organ bath.

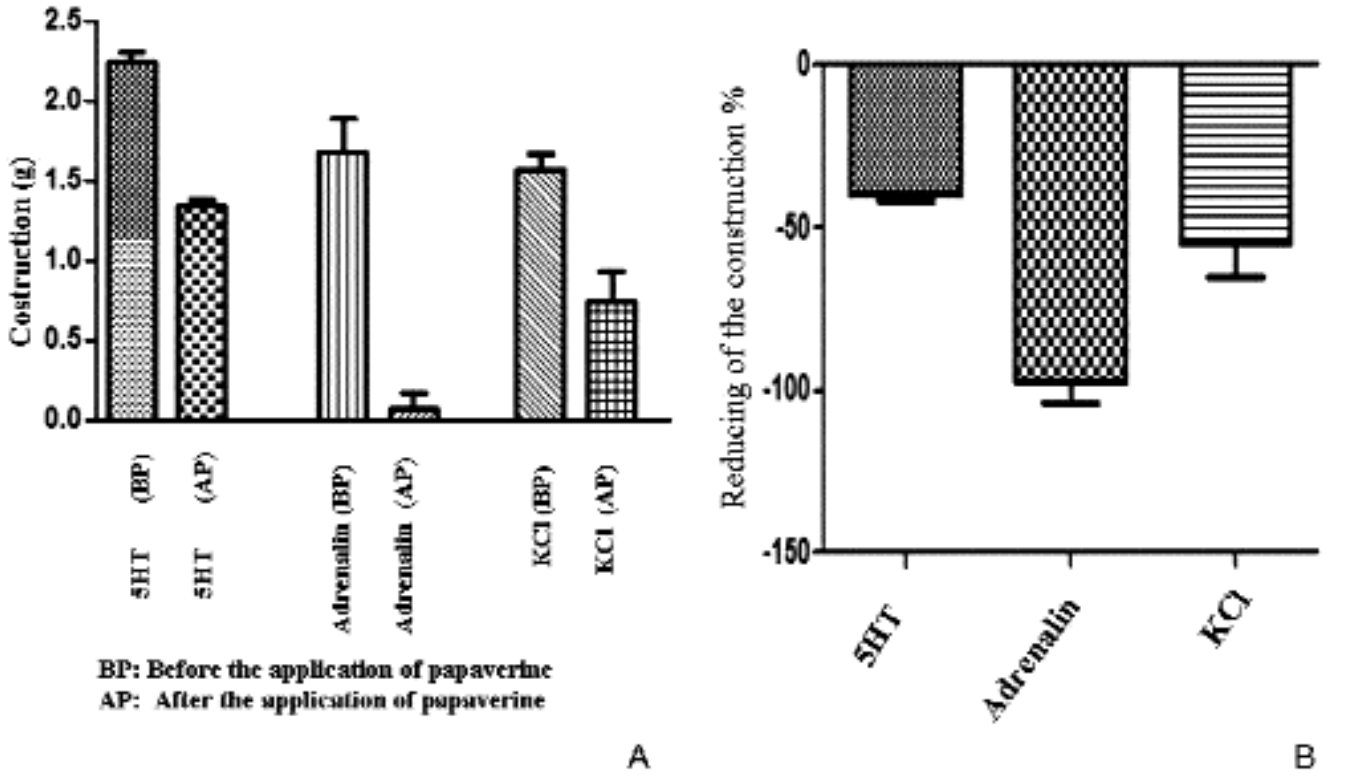

Figure-2: A) Comparison of the responses of agents before and after the papaverine application. B) The reducing rates in the construction respond of chemicals after the papaverine application.

statistically similar response (p=0.064).

Discussion

Papaverine is a vasodilator with proven efficacy, and has been used clinically for many years. This nonspecific vasodilator agent blocks the distribution of cyclic nucleotides such as cyclic adenosine monophosphate and cyclic guanosine monophosphate and causes vasodilatation by inhibition of phosphodiesterase.[7] Papaverine also shows a relaxation effect in other ways such as by inhibition of release of stored calcium or reduction of calcium influx. Therefore, this agent shows systemic effects as well as localized impacts.[3]

The efficacy of papaverine was previously demonstrated in internal mammalian artery segments constricted with various vasoconstrictor agents, and a concentration-dependent relaxation activity was observed.[3] Jett et al. compared the vasodilator efficacies of nifedipine and papaverine in vascular structures constricted with norepinephrine and potassium. At the end of the observation period, they reported that although niphedipine is a more potent vasodilator, papaverine produced a more significant relaxation of the constricted tissues.[8] At high doses, papaverine can exert a cytotoxic effect, in addition to a strong vasodilation response.[9] Another recent study comparing the effects of levosimendan and papaverine reported that, despite the artery specific vasodilator behaviours of levosimendan, papaverine shows generalized vasodilator efficacy in both artery and venous tissues.[10] Many studies have compared the effects of papaverine and different chemicals in vascular tissues constricted with adrenalin, noradrenalin, 5HT and KCl.[3,10,11] However, few studies have investigated the relationship between the various vasoplegic behaviours of papaverine against in these constricted tissues.

Different vasoconstriction models have been created and different chemicals investigated in organ bath studies.[12,13] This method is widely used in these types of studies because the results obtained are realistic and repeatable.[12,13] For this reason, we designed an organ bath environment for the present study to record the contraction and relaxation responses. One of the main features of the drugs investigated here is their temporary efficacy. We think that permanent effects arise due to the high macromolecular affinity of a drug. The vasoconstriction created with adrenalin was the most

effective condition to show the vasoplegic efficacy of papaverine. This efficacy was only moderately detected with KCl and minimally detected with 5HT. Our results support the hypothesis that different vasoplegic responses can be obtained with papaverine in constriction situations induced for different reasons or with different chemicals.

In summary, the various binding affinities of papaverine to constrictor proteins may be responsible for the different vasoplegic responses seen with different vasoconstrictor agents. We suggest that the previously described mechanisms to explain the vasodilator effects of papaverine account for its action. Papaverine was able to relax the vascular smooth muscle against various agents or conditions in rat aorta and this effect may be associated with the inhibition of phosphodiesterase. However, the mechanism that causes the permanent vasoplegic reaction is still unknown. This chemical agent, which may evoke intraluminal cytotoxic effects, continues to be used periarterially as an effective agent to resolve vasospasm and can be used more effectively if the underlying mechanism of its vaso-relaxant action is clarified.

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