INTRODUCTION

5-hydroxytryptamine (5-HT; serotonin) has physiological and pathophysiological importance due to its effects on the periphery and the central nervous system.[1] In this regard, regulation of the cardiovascular system by 5-HT could result in the complex effects such as hypotension/hypertension, vasodilatation/vasoconstriction, bradycardia/tachycardia primarily depending on which 5-HT receptors are involved.[2] Sumatriptan, a 5-$HT_{1B/1D}$ receptor agonist, is the prototype of triptans used for the acute treatment of acute migraine attacks.[3,4] The therapeutic effect is closely linked with vasoconstriction of cranial blood vessels by the drug [5]. Although sumatriptan has been well tolerated in the acute treatment of migraine attacks, some chest symptoms (i.e. chest pressure, tightness, and pain) mimicking angina pectoris and even myocardial infarction and fatal arrhythmia were reported after the use of sumatriptan.[3,4,6-8] This could be related to the extracranial contractile effects of sumatriptan including coronary vasoconstriction both in vivo and in vitro.[9,10] This effect is thought to be predominantly mediated by agonistic activity of the sumatriptan at 5-HT_{1B} receptors.[6] Myocardial ischemia occurs when coronary blood supply to myocardium is reduced (low-flow or no-flow ischemia), or relative to increased tissue demand (demand ischemia).[11] Reperfusion, that is, the re-admission of oxygen and metabolic substrates together with washout of ischemic metabolites is necessary for the viability of ischemic myocardium. However, reperfusion could also have deleterious effects on ischemic myocardium, the process termed as “reperfusion injury”.[11] Therefore, protection from cardiac ischemia/reperfusion (IR) injury including arrhythmias, myocardial infarction and contractile dysfunction has been the focus of intense research topics. Such a cardioprotective intervention is known as ischemic preconditioning (IPC) which applied brief non-lethal ischemia/reperfusion cycles before sustained ischemia of myocardium.[11]

Previous works have suggested that the pressure rate product (PRP) could be an indirect index of myocardial oxygen consumption.[12, 13] It has been recorded that the tachycardias induced by positive inotropic agents including digitalis glycosides and ouabain enhances myocardial oxygen consumption.[14] As myocardial oxygen consumption increases heart rate is increased and finally it negatively affects the cardiac function.[14]

There are some previous findings showing that sumatriptan can induce an exacerbation of regional myocardial ischemia injury concomitant with a reduction in coronary blood flow.[9] To the best of our knowledge, there is no study to evaluate the effect of sumatriptan on the IPC-induced protection. Therefore, we aimed to investigate the effects of sumatriptan in ischemic conditions in rats subjected to IR.

MATERIALS AND METHODS

Animals

The study was approved by Baskent University Ethical Committee for Experimental Research on Animals (Project no: DA 11/11). 20 Male Wistar Albino rats (250-350g) were used in this study. The rats were housed in cages at room temperature 21±1°C, under 12/12 hours light/dark cycle and were allowed ad libitum access to standard laboratory diet and tap water.

Surgical Procedures

Rats were anesthetized with ketamine/xylazine mixture (60/10 mg/kg, i.p.). Body temperature of rats were measured by a rectal probe and maintained at 37±1 °C with a lamp. In anesthetized rats, tracheotomy was performed for mechanical ventilation through an animal ventilator (Rodent Ventilator 7025 UgoBasile, Italy, 5 mL/100 g, 34 pulse/min room air). A standard limb lead II electrocardiogram (ECG) and heart rate (HR) were continuously monitored and recorded throughout the experiments, using electrocardiograph (ECG 100B; Biopac. System Inc., US) and a computerized data acquisition system. The right juguler vein and left carotid artery were cannulated for administration of sumatriptan (3mg/kg, i.v., bolus injection) and mean arterial pressure monitoring, respectively. The mean arterial blood pressure (MABP) and body temperature were also continuously monitored and recorded throughout the experiment with the same data acquisition system. Before the induction of IR procedures a left thoracotomy was performed through the fourth and fifth intercostal space, and then the pericardium was incised, and the heart was gently exteriorized. Afterwards, ischemia was induced by the occlusion of the left anterior descending artery (LAD) close to its origin. Successful occlusion and ischemia were confirmed by a pronounced decrease in arterial pressure and ECG alteration. At the end of the study, the rats were sacrificed with a high dose anesthetic.

Experimental Protocols

Based on previously published studies, the dose of sumatriptan (3 mg/kg) was selected.[15,16]

Rats were divided randomly into the groups as follows (n=5/per group): Ischemia-reperfusion group (IR): Following a stabilization period of 30-min, the rats were subjected to 10-min of ischemia followed by 10-min of reperfusion (Figure 1).

Sumatriptan ischemia-reperfusion group (SUM-IR): Following a stabilization period of 10-min, a bolus sumatriptan injection (3 mg/kg) was administered. 20 minutes after the sumatriptan injection, the rats were subjected to 10-min of ischemia followed by a 10-min of reperfusion (Figure 1).

Ischemic preconditioning group (IPC): Following a stabilization period of 10-min, IPC was applied by 2 cycles of 5-min ischemia/5-min reperfusion.[17] Afterwards, the rats were subjected to 10-min of ischemia followed by a 10-min of reperfusion (Figure 1).

Sumatriptan ischemic preconditioning group (SUM-IPC): Following a stabilization period of 10-min, a bolus sumatriptan injection (3 mg/kg) was performed and immediately after IPC was applied by 2 cycles of 5-min ischemia/5-min reperfusion. Afterwards, the rats were subjected to 10-min of ischemia followed by a 10-min of reperfusion (Figure 1).

Measured and Calculated Parameters

Hemodynamic variables (MABP, HR) were monitored and recorded to calculate rate pressure product (PRP= MABP × HR / 1000) as an indirect index of myocardial oxygen consumption.[12,13] PRP was calculated after the surgical procedure (baseline), before and at the end of ischemia, at the beginning and end of the reperfusion.

The arrhythmia parameters were also evaluated from the ECG recordings of the rats in accordance with the Lambeth conventions at the end of the experimental protocols.[18] The incidence of VT and ventricular fibrillation (VF) were determined in each group.

Statistical Analysis

Data are expressed as mean ± standard error and the percentage of the incidence. Data of PRP were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test (for selected columns). Incidence of arrhythmia was evaluated by Fisher's exact test. All analyses were carried out using the GraphPad

Prism (version 5.00 for Windows, GraphPad Software, San Diego California USA). *P* value of < 0.05 was considered statistically significant.

Drugs

Sumatriptan succinate (GlaxoSmithKline, Turkey) was dissolved in saline.

RESULTS

PRP values, calculated for myocardial oxygen consumption, during experimental protocols were indicated in Figures 2A-E. PRP values in both baseline and before the ischemia did not significantly differ among the groups. However, the PRP value in the SUM-IPC group was significantly lower than that of SUM-IR group at the beginning of the reperfusion (Fig 2D, $p<0.05$). Although there was a tendency to decrease in the PRP value of SUM-IPC group when compared to IPC group, the difference was not significant at the end of the reperfusion (Fig 2E).

Sumatriptan produced a significant reduction in the incidence of VT in the SUM-IR and SUM-IPC groups (40%). In the IPC group, the incidence of VT was significantly lower than SUM-IPC group (10% and 40%, respectively, $p<0.05$) (Figure 3-A).

The VF was only observed in the IR group and the incidence was 80%. The administration of sumatriptan both in the SUM-IR and in the SUM-IPC groups inhibited the VF similar to IPC group alone (Figure 3-B).

DISCUSSION

We investigated the effect of sumatriptan in myocardial ischemic conditions in anesthetized rats. Our findings showed that sumatriptan is cardioprotective against IR injury, but not as protective as IPC alone. The administration of sumatriptan before IPC resulted in the reduction of myocardial oxygen consumption as shown by decreased PRP at the beginning of the reperfusion. Despite that, it did not provide any additional protection from VT induced by IPC. In addition, administration of sumatriptan alone to the rats subjected to IR in the SUM-IR group produce a reduction in the incidence of VT compared to IR group. Among these groups, the VF was only observed in the IR one. It appears that sumatriptan is effective in preventing VF similar to that of IPC.

IPC has been shown to decrease the ischemia-induced arrhythmias in normal hearts.[19] In consistent with these findings, we demonstrated that IPC conferred a marked reduction in arrhythmogenesis as shown by reduction in the incidence of VT

and prevention of VF. Similarly, administration of sumatriptan alone to the rats subjected to IR decreased the incidence of VT. However, it diminishes IPC-induced protection against VT when applied before IPC. Interestingly, at the beginning of reperfusion, the myocardial oxygen consumption in the SUM-IPC group was lower than SUM-IR group. However, it also did not provide any additional protection from VT in the SUM-IPC group.

Taken together, one might think that sumatriptan could interfere with the common mechanisms of IPC. On the other hand, it may also mimic the IPC by leading to coronary vasoconstriction. Support for this conclusion comes from a number of studies in which sumatriptan-induced contractions of coronary arteries have been shown both in vivo and in vitro.[9,10] Therefore, sumatriptan might interfere with the common mechanisms of IPC. The majority of in vivo human angiographic and positron emission tomography studies with sumatriptan have reported very slight to no coronary artery constriction or reduction in myocardial perfusion with no association to ECG changes or anginal symptoms.[20-23] But it was noticed that even modest epicardial coronary constriction could be sufficient to provoke an ischemic event in patients with coronary artery disease.[23] It has been also reported that sumatriptan provoked coronary vasospasm in patients with variant angina but not in control subjects, suggesting the coronary constrictor effect of sumatriptan may be more notable in patients with an ischemic heart disease.[24] For this reason, sumatriptan should be cautiously used in these kind of patients. Additionaly the physicians must be aware when they prescribe sumatriptan for migraine attacks in patients with any cardiovascular symptoms.

Some mechanisms of IPC are associated with the release of some substances such as adenosine, bradykinin, endothelin and endorphins.[25] Some alternative protective mechanisms independent from signal transduction cascades mediated by antioxidant and anti-inflammatory mechanisms are also involved in the IPC-induced protection.[25] Additionally, IPC exerts protection by reduction of myocardial energy demand during ischemia.[26] In the present study, we demonstrated that myocardial oxygen consumption has been decreased both in the IPC and SUM-IPC groups. Furthermore, the myocardial oxygen consumption in the SUM-IPC group was significantly reduced than SUM-IR at the beginning of the reperfusion. Despite that,

the protective effect from VT was similar in both groups but less than IPC group. Taken together, these findings exclude the possibility that neither IPC nor sumatriptan protects the heart against VT by altering oxygen consumption of the myocardium. It could be thought that mechanism other than the decrease in oxygen consumption might contribute to this protection.

In our study, the duration of ischemia is enough to observe ischemia-induced arrythmias, however longer duration is necessary to see the ischemia-induced infarct area in the heart. It would be interesting to investigate whether sumatriptan could be able to decrease the size of infarct area during longer duration of ischemia. In addition, the lack of molecular mechanisms underlying the effects of sumatriptan concerning IPC in this protection is the limitation of the study. We hope that this study will lead future research with sumatriptan in different ischemic conditions .

CONCLUSION

In conclusion, the results of the study show that bolus injection of sumatriptan alone protects the rat heart against IR injury by decreasing the incidence of arrhythmia. Sumatriptan is cardioprotective against arrhythmias in rats subjected to IR injury but not as protective as in IPC. However, the preventive effect of sumatriptan against VF may be predictive for cardioprotection in the ischemic conditions. Further studies are needed to elucidate which mechanisms of IPC interfere with the sumatriptan.

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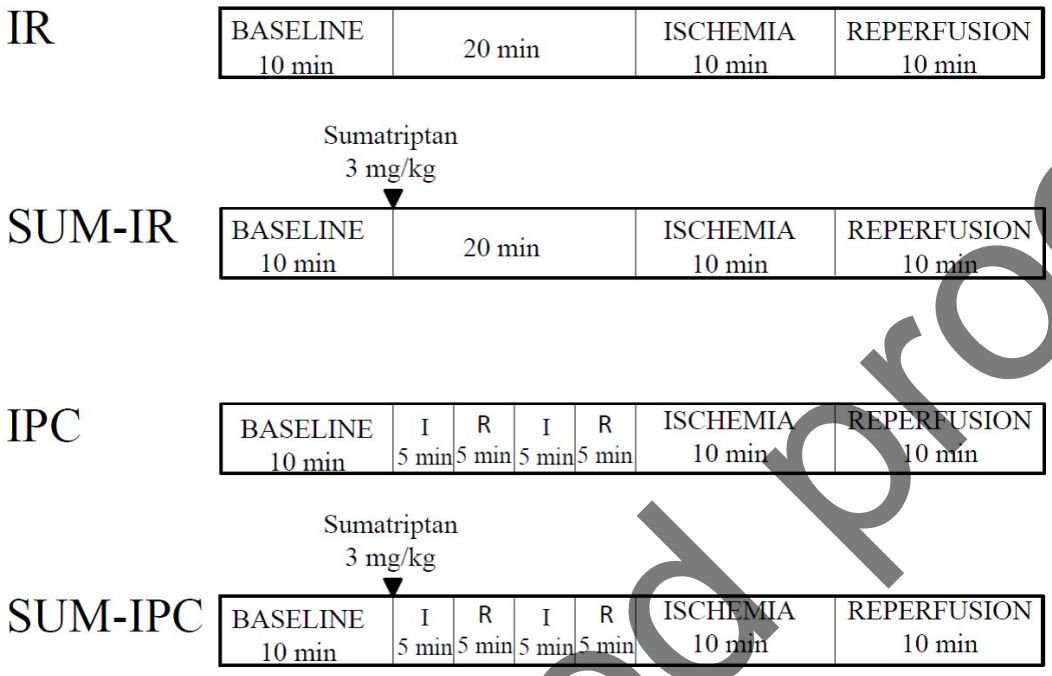

Figure 1. Schematic diagram illustrating experimental protocol. IR: ischemia-reperfusion; SUM: sumatriptan; IPC: ischemic preconditioning; I: ischemia; R: reperfusion.

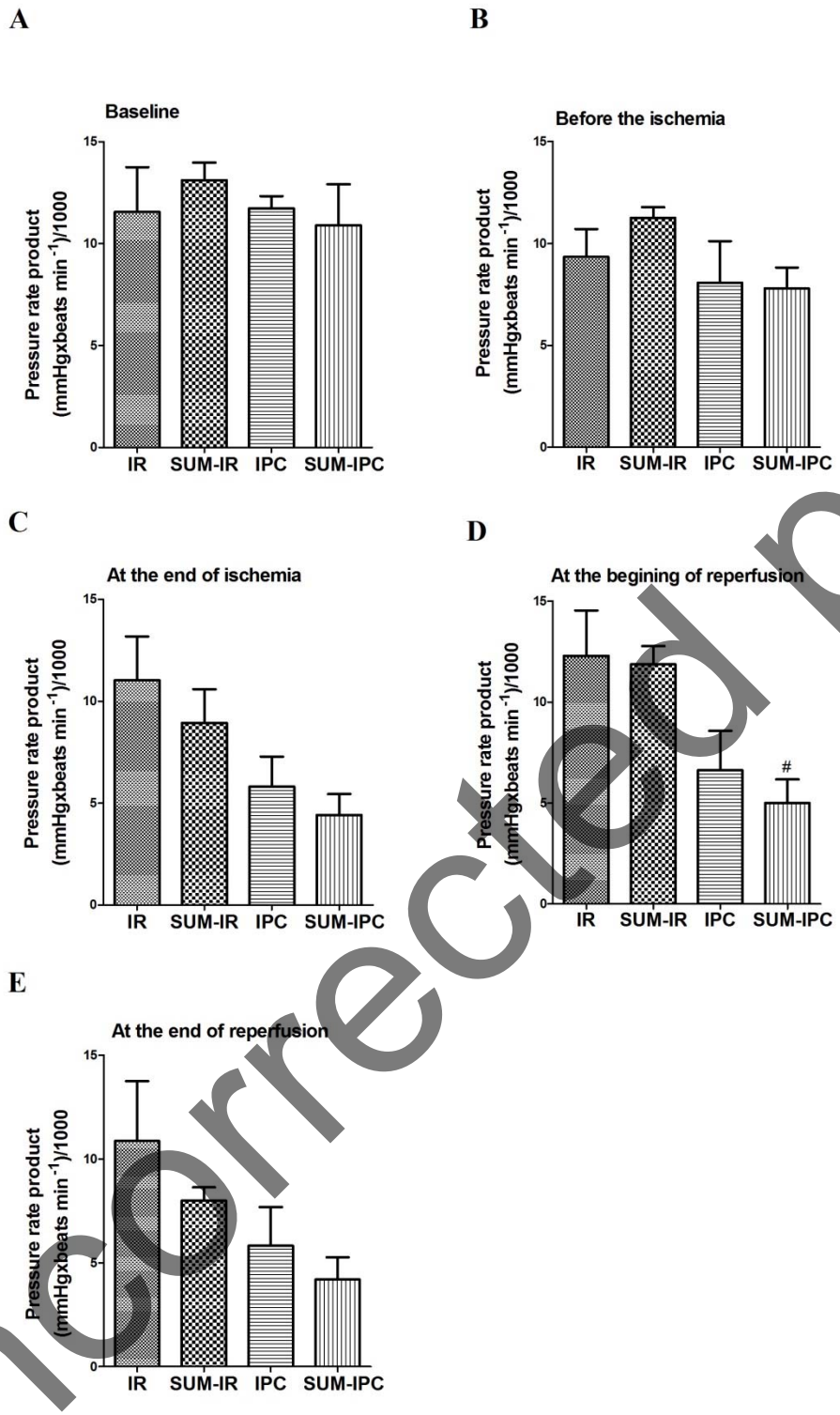

Figure 2. Pressure rate product (PRP) values for baseline (A), before the ischemia (B), at the end of ischemia (C), at the beginning of the reperfusion (D) and at the end of reperfusion (E) in IR, SUM-IR, IPC and SUM-IPC groups. IR: ischemia-reperfusion; SUM: sumatriptan; IPC: ischemic preconditioning. #$p < 0.05$ vs SUM-IR.

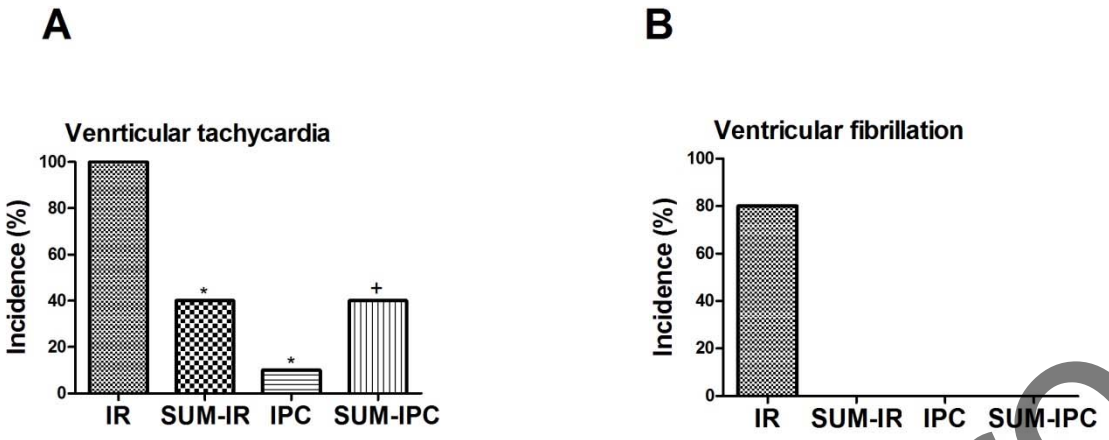

Figure 3. The incidence of ventricular tachycardia and ventricular fibrillation in IR, SUM-IR, IPC and SUM-IPC groups. IR: ischemia-reperfusion; SUM: sumatriptan; IPC: ischemic preconditioning. *p< 0.05 vs IR, ^{+}p<0.05 vs IPC.