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# SCH 79797, a selective PAR1 antagonist, protects against ischemia/reperfusion-induced arrhythmias in the rat hearts

O.Y. MOHAMED[1], A.A. AL-MASRI[2,3], E.A. EL ETER[3,4], R. LATEEF[3]

[1]Department of Pharmacology, King Saud University, Riyadh, Saudi Arabia
[2]Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia
[3]Cardiovascular Research Group, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia
[4]Department of Physiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

**Abstract. – OBJECTIVE: Thrombin is implicated in the genesis of arrhythmias and activation of thrombin receptors exacerbated ventricular arrhythmias following coronary artery ligation. The present study was designed to investigate the possible protective effect of the protease-activated receptor-1 antagonist, SCH79797 against ischemia and reperfusion arrhythmias in the rat heart.**

**MATERIALS AND METHODS: Healthy male Wistar rats (250-350 g) were anesthetized with urethane. Coronary artery ligation was performed for 5 minutes followed by 10 minutes reperfusion. Rhythm disturbances were monitored throughout the ischemia and reperfusion periods. Drugs injected were SCH79797 dihydrochloride (6.25, 12.5, 25 and 100 μg/kg), glibenclamide (5 mg/kg) and N-nitro L-arginine methyl-ester hydrochloride (25 mg/kg). The control group was injected with dimethylsulfoxide (0.1 ml).**

**RESULTS: SCH79797 dihydrochloride reduced the number of premature contraction, prevalence and duration of ventricular tachycardia, prevalence and duration of ventricular fibrillation during both the ischemic and reperfusion periods in a dose-dependent manner. There is a trend for N-nitro L-arginine methyl-ester hydrochloride to increase all parameters of arrhythmias in SCH79797 dihydrochloride (25 μg/kg) treated rats, but glibenclamide (5 mg/kg) significantly ($p < 0.05$) increased these parameters.**

**CONCLUSIONS: SCH79797 dihydrochloride induced an antiarrhythmic effect in the anesthetized rat. This protective effect could possibly be mediated by activation of nitric oxide synthase and/or of ATP-sensitive potassium channels.**

*Key Words:*
Glibenclamide, L-NAME, Heart rate.

## Introduction

Thrombin induced arrhythmias during perfusion of rat hearts[1]. Activation of thrombin receptor increased the ratios of ventricular arrhythmia duration to infarct size, whereas the thrombin antagonist, hirudin significantly reduced the ratio of ventricular arrhythmia durations to infarct size following coronary artery ligation[2]. Moreover, thrombin activated voltage gated sodium channels and enhanced ischemic sodium loading and injury in human cardiomyocytes[3]. Furthermore, thrombin induced persistent sodium current via activation of protease-activated receptor-1 in human cardiomyocytes[4]. The present study was designed to investigate the possible protective effect of the protease-activated receptor-1 antagonist, SCH79797 against ischemia and reperfusion arrhythmias in the rat heart. An attempt was also made to explore the mechanism of antiarrhytmic effect of SCH79797 using glibenclamide and N-nitro L-arginine methyl-ester hydrochloride.

## Materials and Methods

Coronary artery ligation was performed in anesthetized rats by a method similar to that described by Manning et al[5]. Male rats of the Wistar strain (250-350 g body weight) were anesthetized with urethane (1.2 g/kg body weight, IP). The trachea was cannulated for artificial ventilation. Rats were ventilated with room air, using a stroke volume of approximately 10 ml/kg body weight and at a rate of 56 strokes/min (Harvard small animal respiration pump; Harvard Apparatus Ltd. Holliston, MA, USA). The body temperature of the animals was maintained at 37.5°C with a heated surgical



*Corresponding Author:* Abeer A. Al-Masri, MD; e-mail: abelmasri@gmail.com

table and a lamp. The temperature was continuously monitored with a rectal thermometer. A standard limb lead II electrocardiogram (ECG) was recorded throughout the experiment using a Power Laboratory (AD instruments, Bishops Mews, Oxford, UK) data acquisition system via Harvard channel interface adaptor (Harvard Apparatus Ltd., Holliston, MA, USA). The signal from ECG was used to record heart rate.

The chest was opened by a left thoracotomy, an incision was made between the fourth and fifth rib and the heart was gently exteriorized by pressure on the abdomen. A ligature was passed around the left coronary artery and the two ends of the ligature were passed through a polythene tube. The heart was then gently replaced back in the chest, and the rat was allowed to stabilize for five minutes. Ischemia was induced for 5 minutes by clamping the ligature with a bulldog clip. Reperfusion was achieved by removing both the tube and the clip. Hearts were reperfused for 10 minutes. Any animal that developed spontaneous arrhythmias prior to coronary artery ligation was excluded from the study. Rhythm disturbances were monitored throughout the ischemia and reperfusion periods.

Parameters monitored were: premature ventricular contractions (PVCs), percentage prevalence and duration of ventricular tachycardia (VT), percentage prevalence and duration of ventricular fibrillation (VF) and percentage mortality attributable to ventricular fibrillation.

SCH79797 dihydrochloride (Tocris bioscience, Bristol, UK) (6.25, 12.5, 25 and 100 $\mu$g/kg) was dissolved in dimethylsulfoxide and injected intraperitoneally 15 minutes before ligation. Glibenclamide (ICN Biomedicals, Irvine, CA, USA) (5 mg/kg) was injected intraperitoneally 45 minutes before ligation and N-nitro–L-arginine methyl-ester hydrochloride (L-NAME, Sigma Chemical Co., St. Louis, MO, USA) (25 mg/kg) was injected intraperitoneally 30 minutes before ligation. Glibenclamide is an ATP-sensitive potassium channels ($K_{ATP)}$ blocker and L-NAME is a nitric oxide synthase (NOS) blocker. Control group was injected with 0.1 ml dimethylsulfoxide 15 minutes before ligation. Fifteen rats were used in each group.

### Statistical Analysis

All values are represented as mean ± SEM or percentage incidence. 2 analysis was used to test the data on the prevalence of VT, VF using the Yates correction. The non parametric Wilcoxon Rank Sum test was used to compare drug-treated values, with those of controls, of the number of PVCs, duration of VT and VF. Paired *t*-test was used to assess heart rate data. Statistical significance was set at the level of $p < 0.05$.

## Results

### Effects of SCH79797 on Ischemia and Reperfusion-Induced Rhythm Disturbances

It was shown by Manning and Hearse[6] that a 5-minute period of ischemia produced the highest incidence of rhythm disturbances, and all major reperfusion-induced arrhythmias had occurred within a 10-minutes reperfusion period. Therefore, we chose to occlude the coronary artery for 5 minutes and reperfuse the myocardium for 10-minutes.

Table I depicts that all doses of SCH79797 suppressed the number of premature contractions and reduced the prevalence of ventricular tachycardia ($p < 0.05$).

Effects on the prevalence and duration of fibrillation were not statistically significant because the control values were low.

**Table I.** Effect of SCH79797 on ischemia-induced arrhythmias in the anesthetized rats.

| Group | Number of PVC's | Prevalence of VT (%) | Duration of VT (sec) | Prevalence of VF (%) | Duration of VF (sec) |
|---|---|---|---|---|---|
| Controln (n = 15) | 149 ± 18 | 67 | 7 ± 2 | 20 | 5 ± 2 |
| SCH79797 (6.25 $\mu$g/kg) (n = 15) | 31 ± 15* | 20 | 2 ± 1 | 0 | 0 |
| SCH79797 (12.5 $\mu$g/kg) (n = 15) | 27 ± 11* | 7* | 1 ± 1* | 0 | 0 |
| SCH79797 25 $\mu$g/kg (n = 15) | 30 ± 12* | 7* | 2 ± 1 | 0 | 0 |
| SCH79797 (100 $\mu$g/kg) (n = 15) | 35 ± 15* | 7* | 2 ± 1 | 0 | 0 |

PVC's = number of premature ventricular contractions; VT = ventricular tachycardia; VF = ventricular fibrillation. *$p < 0.05$. The number of animals is written in parenthesis.

Reperfusion-induced arrhythmias were more severe than ischemia- induced arrhythmias. Therefore, 67% of the control group exhibited ventricular fibrillation and consequently 20% died (Table II). SCH79797 reduced the duration of ventricular tachycardia at 25 and 100 $\mu$g/kg doses ($p < 0.05$). The prevalence and duration of ventricular fibrillation were both significantly reduced. The levels of significance were $p < 0.05$ and $p < 0.01$ respectively. No animal died when treated with 25 or 100 $\mu$g/kg of SCH79797.

### Effects of SCH79797 on Heart Rates

In the dimethylsulfoxide-treated control animals the heart rate was 384 ± 14 beats per minute. The different drug concentrations did not produce any significant effect on heart rates (Table III).

### Effects of Glibenclamide and L-NAME on Ischemia and Reperfusion-Induced Rhythm Disturbances in Rats Treated with SCH79797

Injection of glibenclamide (45 minutes before ligation of the coronary artery) and L-NAME (30 minutes before ligation of the coronary artery) into rats treated with 25 $\mu$g/kg of SCH79797 did not produce any significant effect on cardiac rhythm during the 5 minutes of coronary occlusion (Table IV). During period of reperfusion L-NAME induce a trend to increase all parameters of arrhythmias but did not reach level of statistical significance. Whereas administration of glibenclamide reduced the prevalence and duration of ventricular tachycardia and fibrillation significantly ($p < 0.05$) (Table V).

**Table II.** Effect of SCH79797 on reperfusion- induced arrhythmias in the anesthetized rats.

| Group | Number of PVC's | Prevalence of VT (%) | Duration of VT (sec) | Prevalence of VF (%) | Duration of VF (sec) | Mortality (%) |
|---|---|---|---|---|---|---|
| Control (n = 15) | 384 ± 82 | 73 | 25 ± 7 | 67 | 112 ± 66 | 20 |
| SCH79797 (6.25 $\mu$g/kg) (n = 15) | 322 ± 80 | 80 | 25 ± 7 | 53 | 232 ± 88 | 40 |
| SCH79797 (125 $\mu$g/kg) (n=15) | 170 ± 36 | 67 | 9 ± 3 | 20 | 84 ± 62 | 20 |
| SCH79797 (25 $\mu$g/kg) (n=15) | 139 ± 48* | 20 | 5 ± 3* | 0* | 0** | 0 |
| SCH79797 (100 $\mu$g/kg) (n=15) | 161 ± 78 | 20 | 6 ± 5* | 0* | 0** | 0 |

PVC's = number of premature ventricular contractions; VT = ventricular tachycardia; VF = ventricular fibrillation. *$p < 0.05$; **$p < 0.01$.

**Table III.** Effect of different concentrations of SCH79797 on heart rates of anesthetized rats.

| | Control | SCH79797 (6.25 µg/kg) | SCH79797 (12.5 µg/kg) | SCH79797 (25 µg/kg) | SCH79797 (100 µg/kg) |
|---|---|---|---|---|---|
| Heart rate b/m | 384 ± 14 | 387 ± 16 | 359 ± 28 | 369 ± 14 | 378 ± 23 |

**Table IV.** Effect of Glibenclamide and L-NAME on ischemia-induced arrhythmias in the anesthetized rats treated with 25 $\mu$g/kg of SCH79797.

| Group | Number of PVC's | Prevalence of VT (%) | Duration of VT (sec) | Prevalence of VF (%) | Duration of VF (sec) |
|---|---|---|---|---|---|
| SCH79797 (25 $\mu$g/kg) (n = 15) | 64 ± 41 | 7 | 2 ± 2 | 0 | 0 |
| Glibenclamide (5 mg/kg) + SCH79797 (25 $\mu$g/kg) (n = 15) | 65 ± 35 | 20 | 2 ± 2 | 0 | 0 |
| L-NAME (25 mg/kg) + SCH79797 (25 $\mu$g/kg) (n = 15) | 10 ± 4 | 0 | 0 | 0 | 0 |

**Table V.** Effect of Glibenclamide and L-NAME on reperfusion-induced arrhythmias in the anesthetized rats treated with 25 $\mu$g/kg of SCH79797.

| Group | Number of PVC's | Prevalence of VT (%) | Duration of VT (sec) | Prevalence of VF (%) | Duration of VF (sec) |
|---|---|---|---|---|---|
| SCH79797 (25 $\mu$g/kg) (n = 15) | 139 ± 48 | 20 | 5 ± 3 | 0 | 0 |
| Glibenclamide (5 mg/kg) + SCH79797 (25 $\mu$g/kg) (n = 15) | 218 ± 82 | 80* | 14 ± 5* | 60* | 119 ± 45* |
| L-NAME (25 mg/kg) + SCH79797 (25 $\mu$g/kg) (n = 15) | 198 ± 68 | 60 | 8 ± 4 | 33 | 61 ± 35 |

PVC's = number of premature ventricular contractions; VT = ventricular tachycardia; VF = ventricular fibrillation. *$p < 0.05$.

### *Effects of Glibenclamide and L-NAME on Heart Rate*

L-NAME has no effect on heart rate but glibenclamide reduced significantly ($p < 0.05$) the heart rate of anesthetized rats (Table VI).

## Discussion

The present study shows that the administration of various doses of SCH79797, a selective PAR1 antagonist, reduced both the prevalence and severity of ventricular arrhythmias during ischemic and reperfusion periods in anesthetized rats. SCH79797 also reduced mortality. Thrombin has been implicated in the genesis of ischemia and reperfusion arrhythmias. Perfusion of isolated rat hearts with thrombin increased arrhythmias during reperfusion[1]. This arrhythmogenic effect of thrombin was attributed to the release of Ins[1,4,5] P3. Injection of thrombin receptor-activating peptide increased the ratios of ventricular arrhythmias (VA) duration to infarction sizing in a coronary artery-ligated rat hearts, while hirudin, the direct thrombin antagonist, significantly reduced the ratio of VA durations to infarction sizing[2]. In the previous study, an increase in activation and expression of thrombin was demonstrated[2].

Thrombin exerts multiple actions that favour the genesis of arrhythmias. It facilitates voltage-gated sodium channel activation in human cardiomyocytes, which may promote ischemic sodium loading and injury[3]. The activation of sodium current is believed to be mediated by PAR1 receptors[4]. Thrombin receptor activating peptide, SFLLRNPNDKYEPF, increased intracellular $Na^+$ during myocardial ischemia in an isolated, blood- perfused rabbit papillary muscle preparation[7]. Activation of thrombin receptor also increased intracellular calcium in rat ventricular myocytes[8] and increased cardiomyocyte acute cell death after ischemia and reperfusion[9].

The limitation of ischemia-reperfusion injury in the rat hearts by SCH79797 was accompanied by activation of nitric oxide synthase (NOS) and ATP-sensitive potassium channels ($K_{ATP}$)[10]. Therefore, we used L-NAME and glibenclamide in order to block the activation of NOS and $K_{ATP}$ channels respectively.

Glibenclamide and L-NAME reversed the protective effect of SCH79797 against ischemia and reperfusion arrhythmias in the present study. Nitric oxide modulates the activity of cardiac ion channels, implicated in the genesis of the cardiac action potential and exerts anti-arrhythmic properties, while NOS inhibitors reduce the coronary effluent NO levels and increase the incidence and severity of ventricular arrhythmias in rat models of ischemia-reperfusion[11]. Hence, in the present work, L-NAME attenuated the antiarrhythmic effect induced by SCH79797. Activation of $K_{ATP}$

**Table VI.** Effect of Glibenclamide and L-NAME on heart rate of rats treated with 25 $\mu$g/kg of SCH79797.

| | SCH79797 (25 µg/kg) | Glibenclamide (5 mg/kg) + SCH79797 (25 µg/kg) | L-Name (25 mg/kg) + SCH79797 (25 µg/kg) |
|---|---|---|---|
| Heart rate b/m | 369 ± 14 | 329±19* | 352 ± 15 |

*$p < 0.05$.

channels exerts opposing effects on the heart. Activation of mitochondrial $K_{ATP}$ channels protects the heart from damage during ischemia by limiting calcium entry[12]. On the other hand, activation of sarcolemmal $K_{ATP}$ channels shortens the action potential and reduces the refractory period favouring the genesis of arrhythmias[13]. Thus, mitochondrial $K_{ATP}$ channel opening and sarcolemmal $K_{ATP}$ channel blockage conferred protection against ischemia-reperfusion-induced arrhythmia in anesthetized male rats[14].

Glibenclamide is a non-selective $K_{ATP}$ channel blocker, it can block both the mitochondrial and sarcolemmal $K_{ATP}$ channels but it seems that its action on mitochondrial $K_{ATP}$ channels prevailed over mitochondrial $K_{ATP}$ channels in the present study.

## Conclusions

Thrombin induces arrhythmias, which are mediated by PAR1 receptor. SCH79797, a potent, selective non-peptide PAR1 receptor antagonist, attenuated these arrhythmias. The antiarrhythmic effect of SCH79797 involves activation of nitric oxide synthase and ATP-sensitive potassium channels. Inhibition of nitric oxide synthase and ATP-sensitive potassium channels by L-NAME and glibenclamide respectively, reversed the protective effect of SCH79797. This suggests a potential therapeutic role for SCH79797 for treatment of ischemia-reperfusion arrhythmias. SCH79797 is endowed with additional privileges. It is antithrombotic, it limits ischemia-reperfusion injury[10] and it attenuates left ventricular remodelling[15], which renders SCH79797 a unique candidate for cardio-protection.

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### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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