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ORIGINAL ARTICLE

# The effects of serotonin on the electrophysiological properties of atrioventricular node during an experimental atrial fibrillation

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**Abstract** A few studies explored the atrioventricular (AV) nodal effects of 5-hydroxytyptamine (serotonin, 5-HT) during supraventricular tachyarrhythmia. The aims of the present study are to investigate (i) 5-HT effects on the rate-dependent electrophysiological functions of AV node during atrial fibrillation (AF) and (ii) the potential contribution of various 5-HT receptors and the role of the autonomic nervous system on 5-HT effects on AV nodal properties. The specific stimulation protocols were applied to detect the electrophysiological parameters of AV node in seven groups of isolated rabbit AV nodal preparations ($N$=75) in the presence of 5-HT (0.5, 1, 5, 10, and 20 μM) and its receptor antagonists, nadolol and atropine. The simulated AF protocol was executed in a separate group, and specific indices, including mean His–His interval, a zone of concealment (ZOC), and concealed beats recorded. 5-HT (10–20 μM) increased significantly functional refractory period, Wenckebach cycle length, and excitability index ($p$<0.05). The percentage of gap and echo beats was significantly decreased with increasing 5-HT concentrations ($p$<0.05). Ketanserin and tropisetron increased significantly atrial-His conduction time, effective refractory period, and Wenckebach cycle length ($p$<0.05). 5-HT effects on functional refractory period and Wenckebach cycle length were abrogated by tropisetron and nadolol ($p$<0.05). 5-HT elicited prolongation of ZOC and nodal refractoriness ($p$<0.05). We conclude that 5-HT elicited prolongation of the nodal refractoriness more than atrial-His conduction time leads to increase in the excitability index and ZOC without significant reduction of the ventricular rates during AF.

**Keywords** 5-HT · Atrial fibrillation · Atrioventricular node

**Abbreviations**

| | |
|---|---|
| 5-HT | 5-Hydroxytyptamine |
| AF | Atrial fibrillation |
| AH | Atrial-His conduction time |
| AERP | Atrial effective refractory period |
| AV | Atrioventricular |
| BCL | Basic cycle length |
| ERP | Effective refractory period |
| EC50 | Effective concentration 50 |
| FAC | Facilitation |
| Fatigue index | ΔAHmax |
| FRP | Functional refractory period |
| H–H mean Mean | His–His interval |
| NCT | Nodal conduction time |
| NERP | Nodal refractoriness |
| REC | Recovery |
| WBCL | Wenckebach cycle length |
| ZOC | Zone of concealment |

## Introduction

Atrioventricular (AV) node plays a strategic role in the occurrence of cardiac arrhythmias. Considerable evidence suggests that the dynamic regulation of three intrinsic nodal properties (recovery, facilitation, and fatigue) can explain the diverse and complex nodal behaviors that may be important for regulating a patient's physiology and cardiac arrhythmias (Billette and Nattel 1994). Experimental and clinical data have proved the specific modification of the nodal slow pathway to control ventricular rate during atrial fibrillation (AF) (Bertaglia et al. 2010; Liu et al. 2004). Some studies have shown the role of

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nodal refractoriness and concealed conduction in anticipating ventricular rate during AF (Climent et al. 2011; Xue and Wang 2010). Therefore, further research to identify the effects of the endogenous substances on AV node during experimental AF can be of clinical interest. Evidence over more than four decades has established an expression of 5-hydroxytryptamine (5-HT, serotonin) and its receptors in the sinoatrial node, the left and the right atrium (Leonard 1991; Villalon and Centurion 2007). The presence of 5-HT in the heart suggests its potential involvement in the physiological or the pathological cardiac stresses (Levy et al. 2008). Kaumann and Sanders (1994) suggested that 5-HT released from platelets may precipitate arrhythmias, particularly in the patients chronically treated with β-adrenoceptor antagonist. An increase in reverse rate-dependent arrhythmic activity by 5-HT in the human atrium, acting through 5-$HT_4$ receptors, has been suggested to lead to atrial flutter and AF (Kaumann and Sanders 1994). Clinical evidence supporting this suggestion is rare, although cisapride (a partial 5-HT agonist) can elicit tachycardia in the human atrium by stimulating the sinoatrial 5-$HT_4$ receptor and thereby generating supraventricular arrhythmia (Keller and Di Girolamo 2010).

Given the great similarities between the atrial and the nodal tissues, 5-HT may produce the sympathetic-like effects on the atrioventricular node and thereby generate or accelerate the supraventricular tachyarrhythmia. We hypothesized that the effects of 5-HT on AV node are mediated at least partly by 5-HT and adrenoceptor. Therefore, our goals are to evaluate (i) the potential contribution of 5-HT receptor subtypes on the basic and the rate-dependent electrophysiological properties of AV node; (ii) the potential role of the serotinergic and the adrenergic receptors on 5-HT effects, and (iii) the effect of 5-HT on the electrophysiological properties of AV node during AF.

## Materials and methods

### General methods

All experiments were performed on an isolated heart preparation obtained from male New Zealand white rabbits (1.8–2.8 kg). The preparation, the stimulation technique, and the recording systems were similar to our previous studies (Khori et al. 2012a, b, 2015). All animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996).

### Stimulation protocols

All basic values were calculated using the following specific stimulation protocols, as previously described (Nayebpour et al. 2001; Khori et al. 2012a, b, 2015). Wenckebach cycle length (WBCL), atrial-His conduction time (AH), effective refractory period (ERP), functional refractory period (FRP), and the frequency-dependent nodal variables (recovery, facilitation, and fatigue) were measured as previously explained (Khori et al. 2015). Differences between AH and ERP were measured as an excitability index, which indicated a relative effect of a drug on AH prolongation against refractoriness.

The simulated random high-rate AF was created by the software in 75–125 ms coupling intervals. In each AF protocol, we stimulated 1500 beats over 5 min and recorded the mean, the shortest and longest His–His interval (H–H), and concealed beats.

A concealed conduction and zone of concealment (ZOC) were calculated as previously defined by Khori et al. (2012b). The rate-dependent effects of the ZOC were measured by applying various stimulation cycle lengths (300–160 ms). ZOC was calculated as the difference between atrial refractoriness (AERP) and nodal refractoriness (NERP). In addition, differences between AH and ERP were measured as the excitability index, indicating the drug's relative effect on nodal conduction time prolongation against refractoriness (Khori et al. 2012b, 2015). The AV nodal gap (conduction gap) refers to the duality of the AV node and is defined as a window of each coupling interval, within which the atrial stimulus was not conducted through the AV node. Echo beats (reentry beats), the landmark of a dual pathway, are defined as a single or multiple premature atrial beats that can reenter the AV node during short cycle length stimulation (Khori et al. 2015).

**Table 1** Time course of the stability of preparations during 3-h stimulation protocols

| Times (min) Indices (ms) | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
|---|---|---|---|---|---|---|---|
| AH (ms) | 61.4±2.90 | 64.0±4.20 | 61.80±2.8 | 63.00±2.1 | 63.20±2.2 | 66.2±2.4 | 64.80±2.2 |
| ERP (ms) | 113±12.34 | 99.8±12.4 | 105.4±12.4 | 107.6±9.3 | 112.2±6.9 | 108.2±4.6 | 110.0±8.2 |
| FRP (ms) | 157±05.80 | 158.4±5.1 | 156.0±3.6 | 159.2±2.7 | 158.6±2.7 | 158.8±2.2 | 159.5±1.2 |
| WBCL (ms) | 150.0±5.00 | 151.0±8.9 | 148.0±3.2 | 149±3.7 | 151.0±2.5 | 153.0±2.6 | 153.3±4.4 |

Results are shown as mean±SEM; $n=5$; basic cycle length=300–350 ms

*WBCL* Wenckebach cycle length, *FRP* functional refractory period, *ERP* effective refractory period, *AH* atrial-His conduction time

**Table 2** Effects of Ach (0.5–10 μM) on the electrophysiological properties of AV node

| Group parameters (ms) | Control | Ach 0.5 | Ach 1 | Ach 2 | Ach 3 | Ach 5 | Ach 10 |
|---|---|---|---|---|---|---|---|
| AH | 069.70±6.80 | 67.7±6.3 | 69.0±4.5 | 68.5±4.4 | 69.6±5.4 | 68.0±6.70 | 72.200±7.60 |
| ERP | 101.80±5.30 | 103.8±8.6 | 122.3±4.3 | 101.0±11 | 109.5±8.3 | 110.5±10.3 | 106.80±7.10 |
| FRP | 163.00±5.60 | 161.8±5.8 | 166.8±5.2 | 166.0±5.0 | 167.5±8.2 | 167.0±6.80 | 169.50±9.60 |
| WBCL | 161.30±2.30 | 162.5±3.3 | 161.0±6.3 | 161.3±4.3 | 163.3±7.7 | 166.0±8.10 | 168.00±11.3 |
| SCL | 364.80±16.1 | 383.3±11.3 | 390.0±10.1 | 397.2±11.1 | 410.0±14.9 | 413.5±16.1 | 415.50±14.1 |
| APD90 | 086.70±2.80 | 077.7±2.1 | 078.1±2.6 | 77.5±2.4 | 75.1±1.9 | 73.00±2.30 | 70.200±4.20 |
| APD60 | 054.10±0.70 | 049.9±0.5 | 051.5±2.1 | 51.9±0.8 | 40.5±0.5 | 39.10±1.20 | 39.000±1.30 |
| Triangulation | 044.20±0.50 | 037.8±1.6 | 036.8±0.6 | 37.1±0.8 | 35.1±0.6 | 37.30±0.40 | 37.700±0.20 |

Results are shown as mean±SEM. An extracellular monophasic action potential recording (MAP) obtained from upper part of right atrium; $n$=6; triangulation=AP90−AP30

*APD60* action potential duration 60 %, *APD90* action potential duration 90 %, *SCL* sinus cycle length

## Experimental protocols

Sixty-five rabbits were randomly divided into seven groups. All stimulation protocols were performed within 20 min after 5-HT administration. Seven groups of the experiments were conducted to test

(a) The stability of the isolated heart preparations ($n$=15) and the normal reactivity of tissue to acetylcholine (ACH) and isoprenaline (ISO) were tested.
(b) 5-HT (0.5–20 μM) effects on the AV nodal electrophysiological properties ($n$=12).
(c) The ability of various antagonists to block the effects of 5-HT (10 μM) on nodal properties (five series of experiments, $N$=40).
(d) 5-HT (0.5–20 μM) effects during an experimental AF ($N$=8).

**Table 3** Effects of isoprenaline (0.05–0.1 μM) on the electrophysiological properties of AV node

| Group parameters (ms) | Control | ISO 0.05 | ISO 0.1 |
|---|---|---|---|
| AH | 66.7±6.8 | 52.70±6.3 | 53.5±4.5 |
| ERP | 103.8±4.3 | 99.80±6.6 | 107.3±6.3 |
| FRP | 168.0±5.6 | 166.8±5.8 | 184.8±5.2 |
| WBCL | 167.3±2.3 | 155.5±3.3 | 161.0±6.3 |
| APD90 | 65.30±0.9 | 78.70±1.3 | 60.00±0.2 |
| APD60 | 37.00±0.4 | 44.50±0.6 | 36.90±0.2 |
| Triangulation | 34.80±0.3 | 42.80±0.3 | 30.30±0.4 |
| Sinus cycle length | 355.5±4.1 | 257.5±6.3 | 237.0±7.1 |

Results are shown as mean±SEM. An extracellular monophasic action potential recording (MAP) obtained from upper part of right atrium; $n$=6; triangulation=AP90−AP30

*APD60* action potential duration 60 %, *APD90* action potential duration 90 %, *SCL* sinus cycle length, *ISO* isoprenaline

The preparations were allowed to equilibrate in Krebs and Henseleit buffer for 20 min and then were stimulated at a basic cycle length. We used only those preparations that maintained stable AH and WBCL during this time. The stimulation protocols were (a) control (no intervention), (b) 5-HT receptor antagonists, and (c) 5-HT plus antagonists.

Following antagonists were added to the solutions: ketanserin (5-$HT_2$ receptor antagonist, 1 μM), tropisetron (5-$HT_3$ receptor antagonist, 1 μM), SB203186 (5-$HT_4$ receptor antagonist, 0.2 μM), nadolol (beta-adrenergic antagonist, 1 μM), and atropine (muscarinic receptor antagonist, 1 μM).

The stimulation protocols were repeated 20 min after adding ketanserin and tropisetron and 15 min after adding SB203186, nadolol, and atropine. Thereafter, 5-HT (10 μM) was added to the solution, and the same protocol was repeated within 20 min.

## Exclusion criteria

Data were excluded from the final analysis if AH was greater than 80 ms and WBCL and FRP were greater than 185 ms. Superimposing the smooth and the steep parts of the recovery curve during the first 30 min of the perfused tissue was also one of the fundamental inclusion criteria for preparations.

## Drugs

Nadolol, 5-HT creatinine sulfate, and atropine sulfate were purchased from Sigma Company (St. Louis, Mo, USA). The following drugs were gifts: ketanserin tartrate from Janssen (Beerse, Belgium), (3-tropanyl) 1-H-indole-3-carboxylic acid ester (tropisetron; Novartis, Basel, Switzerland) from Sandoz, and 1-piperidine ethyl 1-H-indole-3-carboxylate (SB203186) from SmithKline Beecham (Harlow, UK). All drugs were dissolved in Krebs and Henseleit buffer (Khori et al. 2015). An exception was ketanserin, which was dissolved in lactic acid (0.04 M) before being added to the superfusion solution.

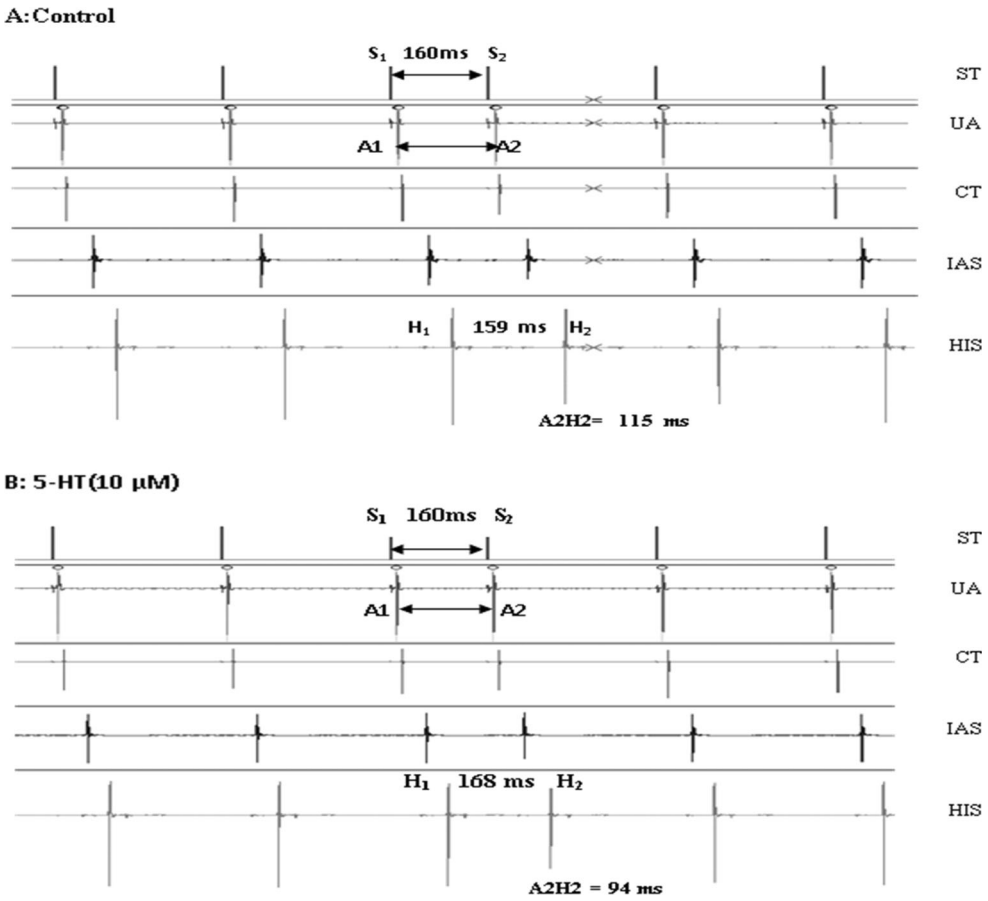


**Fig. 1** Signal electrograms stimulated by bipolar electrode recorded from different parts of the atria and the his bundle of isolated rabbit heart before (**a**) and after (**b**) applying 5-HT (10 μM). Tracings obtained during the last basic stimulation and the test stimulation. *SS* stimulus, *SAN* recording from near sino-atrium node, *CT* crista terminalis, *IAS* intra-atrial septum

## Statistical analysis

All results are presented as mean±SEM. All data were tested for normality before applying the statistical tests. Two groups of experimental data were compared using a Student's *t* test. Comparisons among multiple groups were made by one-way analysis of variance with Scheffe contrasts. Non-linear curve fitting was performed with Marquardt's technique using a computer. A probability of 5 % or less was considered to indicate the statistical significance.

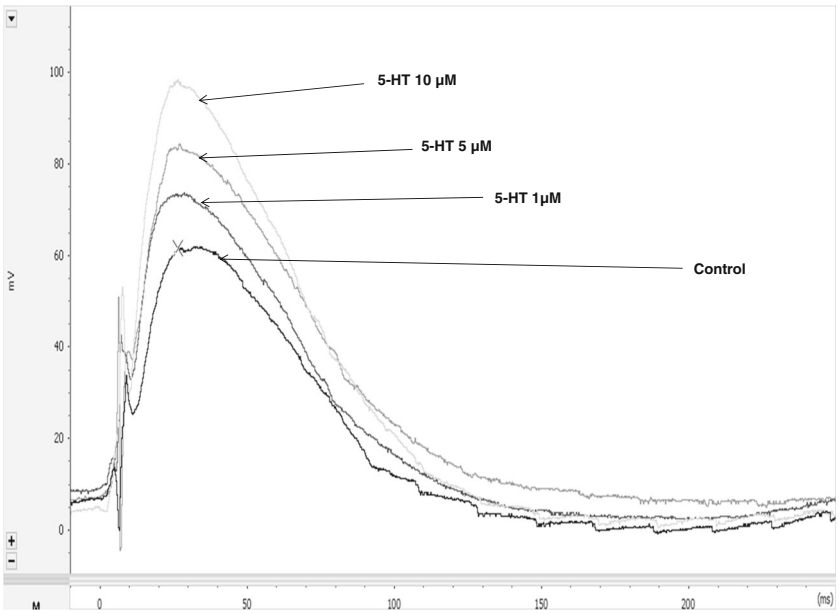


**Fig. 2** Effect of 5-HT (1–10 μM) on the extracellular and the monophasic action potential (MAP) recording from several spots from the rabbit atrial and the nodal tissue

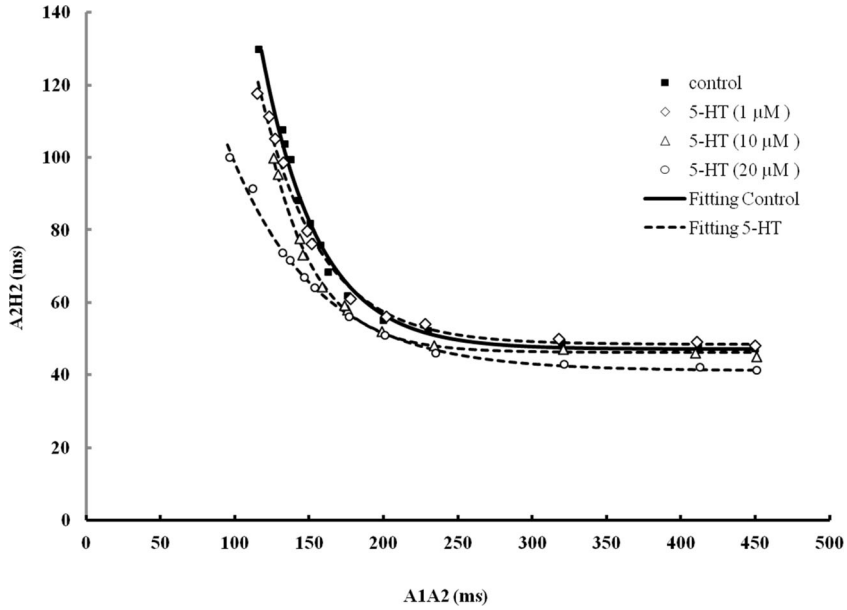


**Fig. 3** Plot of 5-HT effects on the recovery curve in a representative experiment with a rabbit preparation in the presence of 5-HT. 5-HT shifted steep parts of recovery curve and disrupt of the curve. *5-HT* serotonin, *A2H2* AV conduction time, *A1A2* recovery interval

## Results

The stability of the preparations during the experiment was assessed in a separate group ($n$=5) by the repeating stimulation protocols every hour over 180 min in the presence of Krebs and Henseleit buffer. In the preliminary study, the normal reactivity of the preparation was tested in the presence of Ach (5–100 μM) and Iso (0.05 and 0.1 μM). A preliminary study showed no significant changes in the nodal parameters during the 180 min stimulation protocols (Table 1). Ach depressed the AV node function in a wide range of concentrations. Additionally, Ach decreased the amplitude and the duration of the monophasic action potential and the extracellular field potential. The spontaneous sinus rhythm decreased by Ach (Table 2) and increased by Iso (Table 3).

Figure 1 depicts the electrograms of a representative experiment obtained during the recovery protocol. All panels illustrate the traces obtained from different spots on the preparation (Fig. 1). The monophasic action potential was recorded from the upper right atrium. 5-HT increased the amplitude of the action potential and the triangulation, but it did not reach to a significant level (Fig. 2). Figure 3 shows the down-leftward shifts of the recovery curve induced by 5-HT. Each curve was fitted by a single exponential function ($R^2$<0.99). The mean time constant of recovery ($\tau_{rec}$) was decreased in response to 5-HT (from 48±3 to 41±4 ms) ($p$>0.05). 5-HT (≥10 μM) increased FRP and WBCL (EC50=1.46 and 1.81 μM, respectively, $p< 0.05$). The maximum effect of 5-HT was seen at 10–20 μM concentrations ($p$<0.05) (Table 4, Fig. 4). 5-HT (10–20 μM) decreased the facilitation index and increased the

**Table 4** The effect of 5-HT (0.5–20 μM) on the basic electrophysiological parameters, gap, echo, maximum facilitation, and fatigue index of AV node during the predefined stimulation protocols

| 5-HT (μM) | $AH_{min}$ (ms) | $AH_{max}$ (ms) | FRP (ms) | ERP (ms) | WBCL (ms) | Echo (ms) | Gap (%) | Fatigue (ms) | ΔFRP (ms) | Excitable index (ms) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 49.0±3.3 | 133.8±15.1 | 159.3±2.8 | 95.8±8.0 | 134.2±7.5 | 2.5±2.3 | 50 | 24.3±2.4 | 18.1±1.9 | – |
| 0.5 | 48.0±3.7 | 125.5±13.7 | 160.2±2.3 | 99.6±8.0 | 135.8±8.1 | 2.3±1.0 | 66 | 27.9±3.6 | 14.2±3.2 | 4.8±2.1 |
| 1 | 50.0±3.5 | 125.7±10.9 | 163.3±7.8 | 89.3±9.3 | 138.3±7.4 | 2.2±1.6 | 66 | 29.1±4.5 | 13.1±2 | 0.1±0.9 |
| 5 | 51.8±5.8 | 123.5±11.8 | 164.2±7.5 | 108.2±13.8 | 148.3±8.2 | 1.1±1.1 | 33.3 | 32.1±4.8 | 10.4±2.1 | 9.6±4.6* |
| 10 | 49.8±6.3 | 115.8±12.9 | 177.2±5.1* | 112.2±10.1 | 159.3±9.8* | 0.80±0.3* | 11.1* | 33.4±7.2* | 8.7±1.4* | 15.6±7.3* |
| 20 | 53.0±7.7 | 110.4±11.5 | 196.2±9.8* | 110.0±15.4 | 182.3±11.7* | 0.85±0.5* | 8.4* | 35.7±9.1* | 9.3±0.9* | 10.2±9.4* |

All data depicted as mean±SEM. $N$=12; basic cycle length=300–350 ms. Echo beats (reentry beats) are defined as a single or multiple premature atrial beats that can reenter the AV node during short cycle length stimulation. The AV nodal gap (conduction gap) refers to the duality of the AV node and is defined as a window of each coupling interval, within which the atrial stimulus was not conducted through the AV node

*AH* atrial-His conduction time, *WBCL* Wenckebach cycle length, *ERP* nodal effective refractory period, *FRP* nodal functional refractory period, *ms* milliseconds, *5-HT* serotonin, *ΔFRP* facilitation index, *Excitable index (ms)* calculated as differences between ERP and AH at each concentration

*$p$<0.05 compared to control

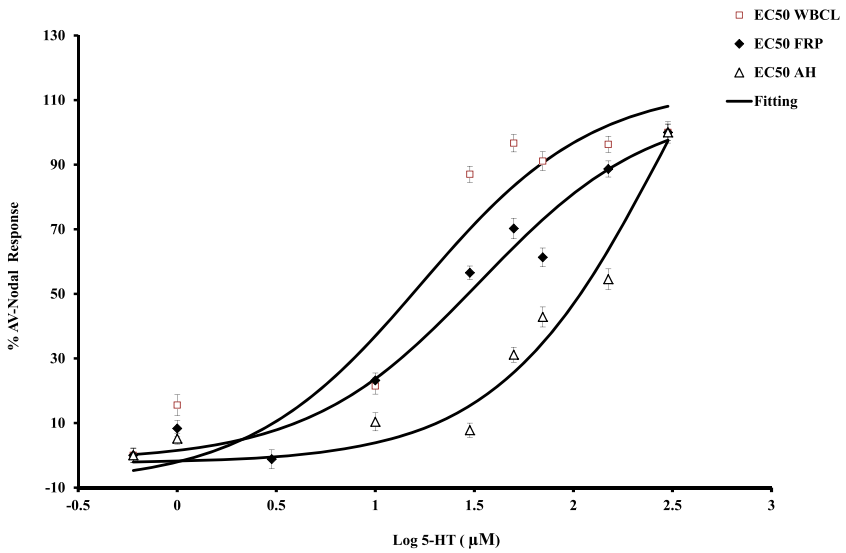


**Fig. 4** The concentration-dependent effects of 5-HT on AV nodal parameters. *5-HT* serotonin, *AH* atrial-his conduction time, *FRP* functional refractory period, *WBCL* Wenckebach cycle length

fatigue index ($p<0.05$) (Table 4). Furthermore, the percentage of the gap and the echo beats was decreased with increasing 5-HT concentrations ($p<0.05$) (Table 4).

Ketanserin and tropisetron increased WBCL and FRP ($p<0.05$), whereas SB203186 had negligible effects (Table 5). Tropisetron, SB203186, and nadolol abrogated 5-HT effects on the nodal refractoriness ($p<0.05$) (Table 5), whereas ketanserin increased this index (Table 6). Ketanserin and tropisetron in the absence of 5-HT increased the fatigue index ($p<0.05$). Ketanserin, SB203186, and atropine did not prevent the effects of 5-HT on the fatigue index, whereas tropisetron and nadolol blocked these effects. Moreover, nadolol and atropine did not change the basic AN node parameters (Table 5), but nadolol abolished the effects of 5-HT on the nodal refractoriness (Table 5), indicating that the effects of 5-HT can mediate by β-adrenergic receptors.

AF protocols were applied at the rate-dependent model to reveal the effects of 5-HT during the different AF rates (Table 7). ZOC was increased with 5-HT (5–20 μM) in a concentration-dependent model (Table 7) by decreasing AERP ($p<0.05$) (Fig. 5). Differences between AH and ERP were measured as excitability index, indicating the drug's relative effect on AH prolongation against refractoriness. The excitability index was increased by 5-HT (5–20 μM) ($p<0.05$) (Fig. 6). Figure 7 shows an example of a two-peak H–H distribution histogram during AF. In the presence of 5-HT, the H–H peak distribution was remained similar to control during AF. In addition, 5-HT eliminated the occurrence of the shortest H–H intervals. Therefore, a control two-peak H–H histogram was transformed into a histogram with one peak in the presence of 5-HT. Moreover, the dominant frequency of the H–H interval during simulated AF was not changed by various concentrations of 5-HT (Fig. 7).

**Table 5** Effects of 5-HT antagonists, nadolol, and atropine on nodal refractoriness parameters in the presence of 5-HT (10 μM)

| | WBCL (ms) | | | FRP (ms) | | |
|---|---|---|---|---|---|---|
| | Control | Antagonist | 5-HT+antagonist | Control | Antagonist | 5-HT+antagonist |
| Ketanserin (1 μM) | 144.2±7.5 | 159.3±9.80* | 176.3±7.80$^{\#}$ | 153.3±6.8 | 168.4±5.7* | 188.1±7.4$^{\#}$ |
| SB203186 (0.2 μM) | 151.8±8.1 | 159.3±9.80 | 179.3±11.8$^{\#}$ | 160.2±5.3 | 162.0±6.4 | 194.5±6.8$^{\#}$ |
| Tropisetron (1 μM) | 138.3±7.4 | 154.3±9.80* | 159.3±10.8 | 163.3±5.8 | 171.6±7.9* | 175.0±9.0 |
| Nadolol (1 μM) | 148.3±8.2 | 153.3±12.2 | 161.3±8.2 | 164.2±6.5 | 170.4±9.9 | 172.4±17 |
| Atropine (1 μM) | 141.3±9.8 | 148.3±8.20 | 165.3±6.2$^{\#}$ | 169.2±5.1 | 174.2±7.1 | 189.0±8.1$^{\#}$ |

Results are shown as mean±SEM, $N=8$

*WBCL* Wenckebach cycle length, *FRP* functional refractory period

*$p<0.05$ compared to control

$^{\#}$ $p<0.05$ compared to antagonist

**Table 6** Facilitation and fatigue indices in the presence of 5-HT (10 μM) and its antagonists and the blockers of adrenergic and muscarinic receptor

| | ΔAH (ms) | | | ΔFRP (ms) | | |
|---|---|---|---|---|---|---|
| | Control | Antagonist | 5-HT+antagonist | Control | Antagonist | 5-HT+antagonist |
| Ketanserin (1 μM) | 18.3±1.5 | 24.9±2.1* | 35.9±2.6$^{\#}$ | 17.7±1.6 | 23.2±1.3* | 11.6±3.4$^{\#}$ |
| SB203186 (0.2 μM) | 20.1±1.6 | 19.6±2.5 | 31.7±2.3$^{\#}$ | 17.8±2.2 | 17.0±3.4 | 15.5±5.8 |
| Tropisetron (1 μM) | 22.0±2.2 | 28.1±3.7* | 29.3±5.2 | 18.0±2.1 | 17.6±2.9 | 8.4±1.4$^{\#}$ |
| Nadolol (1 μM) | 16.5±2.9 | 19.7±3.1 | 23.1±4.7 | 21.1±2.0 | 20.3±3.9 | 22.6±4.3 |
| Atropine (1 μM) | 21.7±1.3 | 22.6±2.4 | 34.7±4.1$^{\#}$ | 17.6±2.6 | 16.2±3.1 | 9.1±1.0$^{\#}$ |

Results are shown as mean±SEM; $N$=9

*ΔAH* fatigue index, *ΔFRP* facilitation index

*$p$<0.05 compared to control

$^{\#}$ $p$<0.05 compared to antagonist

## Discussion

The results of the present study show that 5-HT elicited the prolongation of nodal refractoriness, excitable index, and ZOC without significant reduction of the ventricular rate during AF. These effects may contribute to a reset excitability role of 5-HT during tachyarrhythmia.

In the present study, 5-HT (>5 μM) demonstrated a negligible reduction in AV nodal conduction time, whereas the refractoriness increased significantly by 5-HT. Nevertheless, cardio-excitatory responses to 5-HT are a well known in the atrial and the ventricular tissues of different species and can occur through a wide variety of mechanisms/receptors. Previous studies have proven that the cardiostimulatory effects of 5-HT are mediated via some mechanisms including (8): (a) a tyramine-like action, (b) a direct receptor myocardial effect (cat), and (c) neuronal catecholamine release. An indirect adrenergic-like mechanism for 5-HT has been proposed in the atrial and the ventricular tissues of rabbit and human, operating through 5-$HT_3$ and 5-$HT_4$ receptors (Kaumann and Sanders 1994; Kaumann et al. 1996). Furthermore, 5-HT has been demonstrated to shorten action potentials in the human atrium via L-type $Ca^{2+}$ currents and 5-$HT_4$ receptor (Kaumann and Levy 2006). As previously shown by Kaumann (1994), 5-HT facilitates the induction of the rate-dependent arrhythmic contraction in an isolated atrium through the 5-$HT_4$ receptor subtype (Afzal et al. 2008). In addition, an indirect adrenergic-like mechanism for 5-HT has been proposed in rabbit atrial tissue, operating through 5-$HT_4$ receptors (Kaumann and Sanders 1994). The activation of 5-$HT_3$ receptors on the sympathetic nerve endings mediated the cardiostimulatory effects of 5-HT in rabbit atrium (Saxena and Villalón 1990), whereas 5-$HT_4$ receptor induced the positive inotropic effects in human atrium (Kaumann et al. 1996). Additionally, the activation of 5-$HT_3$ receptor on the sympathetic nerve endings mediated the positive inotropic effects induced by 5-$HT_4$ receptor in the human atrium (Kaumann et al. 1996). Therefore, it seems that our data are consistent with the involvement of serotonin and adrenergic receptors to modulate 5-HT effects on atrioventricular node. In

**Table 7** The effect of 5-HT (0.5–20 μM) on the protective parameters of AV node during atrial fibrillation protocol executed in three different cycle length (fast, medium, and slow)

| | | AF–ERP (ms) | AF–FRP (ms) | H–H max (ms) | H–H mean (ms) | Concealed beats | Triangulation (ms) | APD 90 (ms) | APD 60 (ms) |
|---|---|---|---|---|---|---|---|---|---|
| Control | Slow | 84.5±1.1 | 121.7±5.9 | 585.3±35.4 | 237.5±6.4 | 793.5±20.2 | – | – | – |
| | Medium | 79.10±1.7 | 122.5±4.5 | 596.0±24.4 | 226.5±3.9 | 837.3±11.3 | – | – | – |
| | Fast | 77.30±2.3 | 120.5±3.5 | 532.7±30.4 | 214.2±8.0 | 844.2±25.9 | 44.0±2.4 | 95.8±2.1 | 65.4±3.2 |
| 5-HT (μM) | 1 | 79.5±1.7 | 133.3±5.7 | 551.3±60.5 | 216.8±6.1 | 852.8±26.3 | 46.3±1.7 | 99.2±1.9 | 64.6±6.0 |
| | 10 | 81.5±2.5 | 134.7±5.7 | 552.7±30.0 | 214.2±7.6 | 844.0±28.7 | 43.8±2.5 | 97.6±4.1 | 66.0±3.3 |
| | 20 | 83.5±3.9 | 132.3±4.4 | 550.0±29.5 | 219.8±8.5 | 859.2±36.5 | 47.2±301 | 100.2±7.0 | 67.2±5.1 |

Data are presented as mean±SEM. $N$=8

*AF* atrial fibrillation, *AF–ERP* effective refractory period during the AF protocol, *AF–FRP* the functional refractory period during the AF protocol, *H–H max* maximum interval between two continuous beats from the His bundle, *5-HT* serotonin, *APD60* atrial action potential duration 60 %, *APD90* atrial action potential duration 90 %, *triangulation* differences between APD90 and APD30 %

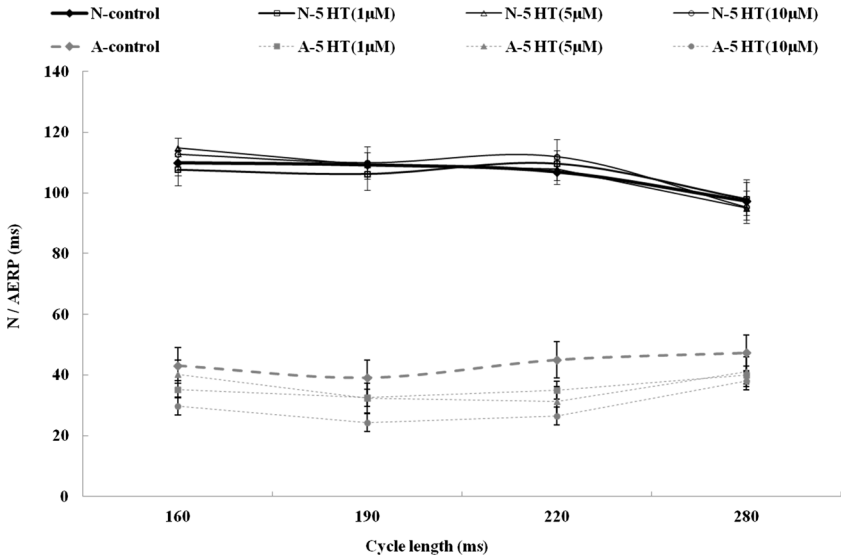


**Fig. 5** Zone of concealment in the presence of 5-HT. Data are presented as mean±SEM. *N* nodal refractoriness (maximum atrial stimulation that cannot conduct through AV node), *A* atrial refractoriness (minimum atrial response during program stimulation protocol). Zone of concealment was measured at the various stimulation cycle length in the presence of 5-HT (1–10 μM). *$p<0.05$ compared to control

the present study, 5-HT induced the prolongation of WBCL and FRP that was prevented by tropisetron and nadolol. Tropisetron may increase FRP and prolong the action potential duration and QT interval due to its direct intrinsic activity via 5-HT receptors. These effects can be related to the inhibitory actions of low concentrations of tropisetron on $I_{Kr}$ and/or its high concentration on $I_{Na}$ and $I_{Ca}$ currents (Scholtysik et al. 1988). Thus, the interplay between adrenergic and 5-HT receptors is a critical point in the interpretation mechanism of 5-HT on the atrio-nodal tissue. In this case, the modulation of the active and the passive membrane properties of the transitional cells of the dual nodal pathways would be the potential target of 5-HT to directly change nodal electrophysiology parameters. Different regional heterogeneity of ionic currents and gap junctions between the atrial and nodal tissue may explain the different electrophysiological activity of 5-HT in these tissues. Further study using the specific $5\text{-HT}_3$/$5\text{-HT}_4$ antagonists is required to determine the role of these receptors in AV node electrophysiology properties.

The present study has shown that 5-HT increase the excitability index without significant change of the ventricular cycle length and ZOC during AF. Ventricular H–H interval response during AF is dependent to basic indices: (1) ZOC and (2) nodal refractoriness. Fundamentally, the ventricular response to AF is the net sum of balance between these intrinsic properties (Liu et al. 2004). In the present study, 5-HT induced the rate-dependent increases in FRP along with ZOC during AF. In addition, the potential modulatory role of 5-HT (10–20 μM) is expressed as an increase in the excitable index and decrease in a gap and echo beats. Taken together, these effects possibly suggest the protective role of 5-HT during tachyarrhythmia. But, the ventricular H–H interval did not affect by 5-HT. On the other hand, the depressant effects of 5-HT prevailed on nodal refractoriness than atrial refractoriness.

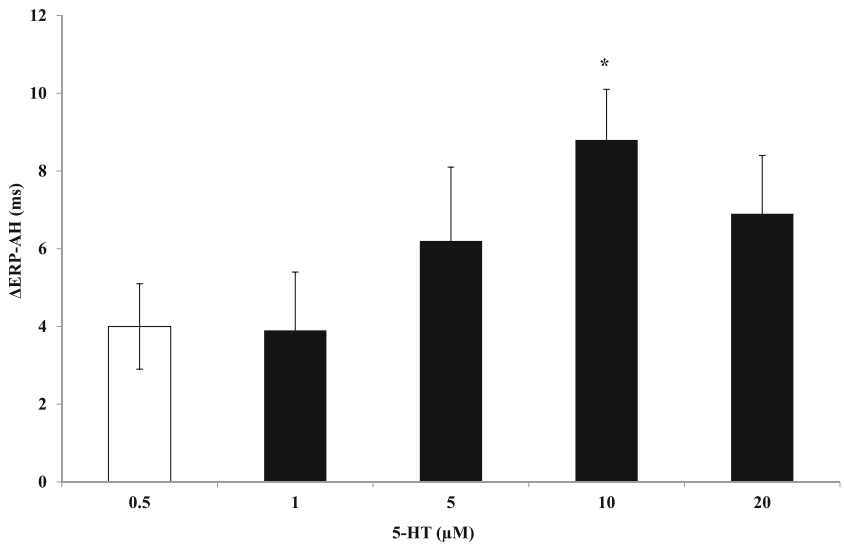


**Fig. 6** Bar graph of changes in the excitability index in the presence of 5-HT. Excitable index defined as the difference between 5-HT-induced increases in the atrioventricular effective refractory period (ERP) and AH interval. Data are presented as mean± SEM; *5-HT* serotonin, *CL* cycle length. All concentrations compared to 0.5 μM 5-HT; *$p<0.05$ compared to 0.5 μM 5-HT

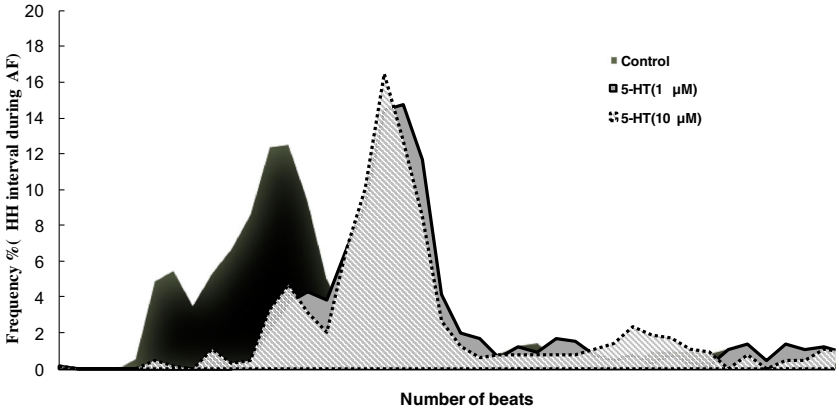


**Fig. 7** Plot of H–H (His–His) intervals recorded during experimental atrial fibrillation model in the presence of 5-HT (1, 5, and 10 μM)

The relationship between ERP and AH determines the functional wavelength of the nodal reentry and the ability of drugs to terminate reentry. Prolonged AH increases the tachycardia cycle length, whereas prolonging ERP terminates the atrioventricular reentry (Khori et al. 2012a, b). Therefore, 5-HT-induced prolongation of the refractory period can prevail over conduction time and can cause increases in the excitable index and also may suggest a mechanism for the anti-arrhythmic effects of 5-HT.

Additionally, a dual nodal pathway has been considered as an anatomical substrate of conduction time and refractoriness (Zhang et al. 2004), which suggests the possibility of 5-HT effects on slow and fast pathways. The interaction between dual pathways could make a nodal recovery curve that include a smooth portion (fast pathway) in the long or the intermediate coupling intervals and a steep portion (slow pathway) in a short coupling interval (Reid et al. 2003). In the present study, 5-HT not only caused an upward shift of the smooth part of the recovery curve, but also, it prolonged the nodal ERP and removed the steep part of recovery curve, which can be explained by the unequal effects on both slow and fast pathways. The reset excitability effect of 5-HT on the dual nodal pathways can be explained by its differential effects on the fast and slow pathways, which manifests as a decrease in the echo beats and gap and an increase in the excitability index. The precise cellular mechanism of 5-HT on atrioventricular conduction time remains unknown. Therefore, the inability of 5-HT to change the ventricular rate possibly can indicate 5-HT other mechanisms. Future studies will be needed to better characterize the molecular electrophysiological mechanism of 5-HT on AV node.

In summary, at both in normal heart rate and during a tachycardia, 5-HT increased the nodal refractoriness more than the nodal conduction time, probably via $5\text{-HT}_3$/$5\text{-HT}_4$ receptors. Furthermore, crosstalk between adrenoceptors and 5-HT receptors are necessary for 5-HT to exert its specific nodal modulatory effects. Differential dominant effects of 5-HT on the nodal dual pathway can lead to increase in the excitability index without the significant protective effects to increase the ventricular cycle length during AF.

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**Conflict of interest** The author(s) declare(s) that they have no conflict of interest to disclose.

## References

Afzal F, Andressen KW, Mørk HK, Aronsen JM, Sjaastad I, Dahl CP, Skomedal T, Levy FO, Osnes JB, Qvigstad E (2008) 5-HT4-elicited positive inotropic response is mediated by cAMP and regulated by PDE3 in failing rat and human cardiac ventricles. Br J Pharmacol 155(7):1005–1014

Bertaglia E, Tondo C, De Simone A, Zoppo F, Mantica M, Turco P, Iuliano A, Forleo G, La Rocca V, Stabile G (2010) Does catheter ablation cure atrial fibrillation? Single-procedure outcome of drug-refractory atrial fibrillation ablation: a 6-year multicentre experience. Europace 12:181–187

Billette J, Nattel S (1994) Dynamic behavior of the atrioventricular node: a functional model of interaction between recovery, facilitation and fatigue. J Cardiovasc Electrophysiol 5:90–102

Climent AM, Guillem MS, Zhang Y, Millet J, Mazgalev TN (2011) Functional mathematical model of dual pathway AV nodal conduction. Am J Physiol Heart Circ Physiol 300(4):1393–1401

Kaumann AJ (1994) Do human atrial 5-HT4 receptors mediate arrhythmias? Trends Pharmacol Sci 15:451–455

Kaumann AJ, Levy FO (2006) 5-hydroxytryptamine receptors in the human cardiovascular system. Pharmacol Ther 111(3):674–706

Kaumann AJ, Sanders L (1994) 5-Hydroxytryptamine causes rate-dependent arrhythmias through 5-HT4 receptors in human atrium: facilitation by chronic beta-adrenoceptor blockade. Naunyn Schmiedebergs Arch Pharmacol 349(4):331–337

Kaumann AJ, Lynham JA, Brown AM (1996) Comparison of the densities of 5-$HT_4$ receptors, beta 1- and beta 2-adrenoceptors in human atrium: functional implications. Naunyn Schmiedebergs Arch Pharmacol 353(5):592–595

Keller GA, Di Girolamo G (2010) Prokinetic agents and QT prolongation: a familiar scene with new actors. Curr Drug Saf 5(1):73–78

Khori V, Alizadeh AM, Yazdi H, Rakhshan E, Mirabbasi A, Changizi S, Mazandarani M, Nayebpour M (2012a) Frequency-dependent electrophysiological remodeling of the AV node by hydroalcohol extract of Crocus sativus L. (saffron) during experimental atrial fibrillation: the role of endogenous nitric oxide. Phytother Res 26(6):826–832

Khori V, Najafi SA, Alizadeh AM, Moheimani HR, Shakiba D, Alizadeh F, Nayebpour M (2012b) Protective role of simvastatin on isolated rabbit atrioventricular node during experimental atrial fibrillation model: role in rate control of ventricular beats. Naunyn Schmiedebergs Arch Pharmacol 385:697–706

Khori V, Alizadeh AM, Moheimani HR, Zahedi M, Aminolsharieh Najafi S, Shakiba D, Nayebpour M (2015) Acute effects of simvastatin to terminate fast reentrant tachycardia through increasing wavelength of atrioventricular nodal reentrant tachycardia circuit. Fundam Clin Pharmacol 29(1):41–53

Leonard BE (1991) 5-Hydroxytryptamine and the human heart. In: Fozard JR, Saxena PR (eds) Serotonin: molecular biology, receptors and functional effects. Birkhauser Verlag, Basel, pp 365–373

Levy FO, Qvigstad E, Krobert KA, Skomedal T, Osnes JB (2008) Effects of serotonin in failing cardiac ventricle: signalling mechanisms and potential therapeutic implications. Neuropharmacology 55(6):1066–1071

Liu S, Olsson SB, Yang Y, Hertervig E, Kongstad O, Yuan S (2004) Concealed conduction and dual pathway physiology of the atrioventricular node. J Cardiovasc Electrophysiol 15(2):144–149

Nayebpour M, Naseri M, Khori V (2001) Effects of ajmaline on rate-dependent atrioventricular node properties. Potential role in experimental atrioventricular re-entrant tachycardia. Eur J Pharmacol 412(1):77–89

Reid MC, Billette J, Khalife K, Tadros R (2003) Role of compact node and posterior extension in direction-dependent changes in atrioventricular nodal function in rabbit. J Cardiovasc Electrophysiol 14(12):1342–1350

Saxena PR, Villalón CM (1990) Cardiovascular effects of serotonin agonists and antagonists. J Cardiovasc Pharmacol 15(7):S17–S34

Scholtysik G, Imoto Y, Yatani A, Brown AM (1988) 5-Hydroxytryptamine antagonist ICS 205-930 blocks cardiac potassium, sodium and calcium currents. J Pharmacol Exp Ther 245(3):773–778

Villalon CM, Centurion D (2007) Cardiovascular responses produced by 5-hydroxytriptamine: a pharmacological update on the receptors/mechanisms involved and therapeutic implications. Naunyn Schmiedebergs Arch Pharmacol 376(1-2):45–63

Xue YZ, Wang LX (2010) Contemporary management of atrial fibrillation: a brief review. Adv Med Sci 55(2):130–136

Zhang Y, Bharati S, Sulayman R, Mowrey KA, Tchou PJ, Mazgalev TN (2004) Atrioventricular nodal fast pathway modification: mechanism for lack of ventricular rate slowing in atrial fibrillation. Cardiovasc Res 61(1):45–55