JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2014 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.

VOL. 64, NO. 19, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.05.077

Atrial Fibrillation Promotion With Long-Term Repetitive Obstructive Sleep Apnea in a Rat Model

Yu-ki Iwasaki, MD, PhD,*† Takeshi Kato, MD, PhD,* Feng Xiong, PhD,*‡ Yan-Fen Shi, MD,* Patrice Naud, PhD,* Ange Maguy, PhD,* Kyoichi Mizuno, MD, PhD,† Jean-Claude Tardif, MD,* Philippe Comtois, PhD,§ Stanley Nattel, MD*‡

ABSTRACT

BACKGROUND Obstructive sleep apnea (OSA) importantly contributes to the occurrence of atrial fibrillation (AF) in humans, but the mechanisms are poorly understood. Experimental research has provided insights into AF promotion by acute OSA episodes. However, patients with OSA usually have frequent nocturnal episodes for some time before manifesting AF.

OBJECTIVES The goal of this study was to test the hypothesis that repetitive OSA causes cardiac remodeling that predisposes to AF.

METHODS We mimicked OSA by using a mechanical ventilator and closing the airway at end-expiration with a 3-way stopcock (OSA rats). Matched control groups included rats with the ventilator stopped but airway left open (open airway rats) and continuously ventilated rats (sham rats). OSA rats were exposed to 20 consecutive 2-min cycles of 40 s of apnea/80 s of ventilation per day, 5 days per week for 4 weeks.

RESULTS OSA significantly increased the duration of AF from (median [interquartile range]) 2.6 s [1.9 s to 8.9 s] (shams) and 16 s [1.8 s to 93 s] (open airway) to 49s [34 s to 444 s]. AF inducibility increased to 56% (9 of 16) of OSA rats; this is up from 15% (2 of 13) and 13% (2 of 15) in open airway and sham rats, respectively ($p < 0.05$). OSA rats exhibited substantial atrial conduction slowing on optical mapping, along with connexin-43 down-regulation on both quantitative immunofluorescence (expression reduced by 58% vs sham rats) and Western blot (reduced by 38%), as well as increased atrial fibrous tissue content (by 71%). OSA also caused left ventricular hypertrophy, dilation, and diastolic dysfunction and enhanced AF inducibility during superimposed acute OSA episodes to 82.4% of rats.

CONCLUSIONS Chronically repeated OSA episodes cause AF-promoting cardiac remodeling, with conduction abnormalities related to connexin dysregulation and fibrosis playing a prominent role. This novel animal model provides mechanistic insights into an important clinical problem and may be useful for further exploration of underlying mechanisms and therapeutic approaches. (J Am Coll Cardiol 2014;64:2013–23) © 2014 by the American College of Cardiology Foundation.

Obstructive sleep apnea (OSA), characterized by repetitive interruption of ventilation during sleep caused by upper airway obstruction, is highly prevalent (1). Recent studies have implicated OSA as a significant risk factor for atrial fibrillation (AF) (2,3). Suggested potential pathophysiological mechanisms include hypoxia (4), negative intrathoracic pressure changes (5),

From the *Department of Medicine, Montréal Heart Institute and Université de Montréal, Montreal, Quebec, Canada; †Department of Cardiovascular Medicine, Nippon Medical School, Tokyo, Japan; ‡Department of Pharmacology, McGill University, Montreal, Quebec, Canada; and the §Department of Physiology, Montréal Heart Institute and Université de Montréal, Montreal, Quebec, Canada. This project was funded by grants from the Canadian Institutes of Health Research (grant 6957) and the Heart and Stroke Foundation of Canada. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.
Listen to this manuscript's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.
You can also listen to this issue's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.

Manuscript received April 1, 2014; revised manuscript received May 8, 2014, accepted May 26, 2014.

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

AV = atrioventricular

ERP = effective refractory period

LA = left atrial

LV = left ventricular

MAPK = mitogen-activated protein kinase

OSA = obstructive sleep apnea

PBS = phosphate-buffered saline

RA = right atrial

RV = right ventricular

sympathovagal imbalance (6), and structural remodeling (7,8). We recently reported that negative intrathoracic pressure changes during acute obstructive apnea promote AF, primarily by causing acute left atrial (LA) dilation (9).

SEE PAGE 2024

Most patients with OSA experience repeated nocturnal OSA episodes. It is conceivable that such repeated events lead to cardiac remodeling. Indeed, patients with repetitive OSA exhibit LA dilation and left ventricular (LV) hypertrophy associated with AF occurrence (10,11). However, the long-term cardiac effects of repetitive OSA on atrial properties and AF susceptibility have not been studied in experimental models. The present study was designed to assess the long-term effects of repetitive OSA on cardiac structure/function and AF susceptibility in a rat model.

METHODS

Only the principal methods are provided here; the Online Appendix provides a detailed description of the methods.

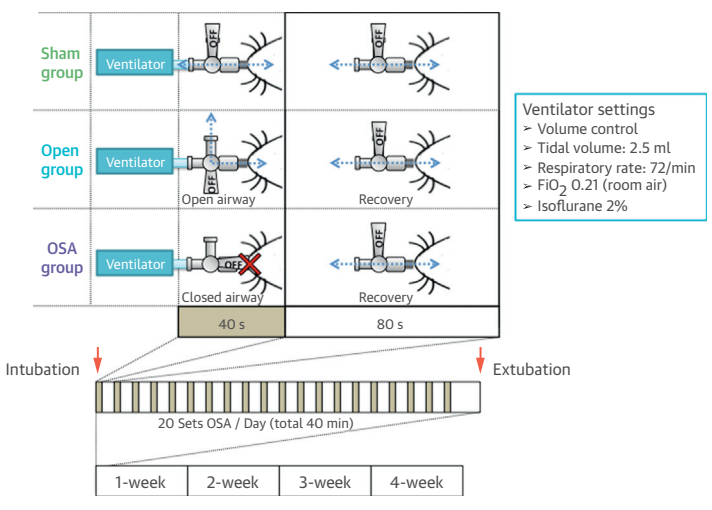

FIGURE 1 Experimental Protocol

Sprague-Dawley rats were ventilated via an endotracheal tube connected to a 3-way stopcock. Obstructive sleep apnea (OSA) was mimicked by stopping the ventilator and closing the airway at end expiration for 40 s, followed by an 80-s recovery period (120 s/cycle). The 2-min cycle was repeated 20 times per day, 5 days per week for 4 weeks. The open airway group had the same cycles but with open (instead of closed) airways during apnea. The sham group was ventilated throughout the procedure. FiO_2 = fraction of inspired oxygen.

LONG-TERM OSA-MODEL. Twelve-week-old male Sprague-Dawley rats were intubated and ventilated with 2% isoflurane/room air. Volume-controlled ventilation was delivered with a tidal volume and respiratory rate of 2.5 ml and 72 cycles/min, respectively. OSA was mimicked by closing the airway at end expiration for 40 s, followed by 80-s recovery periods, 20 consecutive times per day (**Figure 1**). After OSA cycles, rats were ventilated with room air during recovery from anesthesia and then extubated after confirming spontaneous respiration. No complications resulted from anesthesia, ventilation, or OSA. OSA cycles were repeated daily (5 days per week for 4 weeks). Two control groups were studied: 1) open airway rats subjected to the same ventilator-arrest cycles but without airway closure; and 2) sham rats ventilated with isoflurane/room air throughout the procedure. Animals in all 3 groups were subjected to daily oropharyngeal intubation for ventilation. No animal showed signs of complications of intubation such as stridor or tachypnea. After 4 weeks, in vivo electrophysiological studies and AF induction assessments were performed, and tissues were procured.

A 3-F pressure-transducer catheter (Scisense P-catheter; Scisense Inc., London, Ontario, Canada) was introduced into the esophagus close to the LA posterior wall to monitor intrathoracic pressure in 6 OSA rats and 5 open airway rats. For blood pressure monitoring, a 2-F pressure transducer catheter (Scisense P catheter-RAT) was introduced into the ascending aorta through the left internal carotid artery.

IN VIVO ELECTROPHYSIOLOGICAL STUDY. To measure effective refractory periods (ERPs), programmed right atrial (RA) stimulation was performed at a cycle length of 150 ms (pulse width 2 ms, 2 × threshold current). Atrial and atrioventricular (AV) conducting system ERPs were defined as the longest S1-S2 coupling interval failing to generate a propagated beat. To assess atrial tachyarrhythmia inducibility, 25-Hz burst pacing (pulse width 2 ms, 4 × threshold current) was applied for 3 s, with 6 3-s burst cycles separated by 1-s intervals. AF was defined as a rapid (>800 beats/min) irregular atrial rhythm, and AF inducibility was defined as AF lasting for at least 1 s immediately after the 6-burst cycle protocol. If AF was induced after <6 burst pacing cycles, burst pacing was suspended to avoid interfering with the evolution of the AF. AF duration was determined in each rat as the mean duration of all AF episodes. If burst pacing at baseline did not induce AF, the same pacing protocol was repeated during acute OSA. Wenckebach cycle length was defined by failure of