Original Article

A novel system including an N_2 gas generator and an air compressor for inducing intermittent or chronic hypoxia

Hisashi Nagai, Hirotsugu Tsuchimochi[1], Ken-ichi Yoshida[2], Mikiyasu Shirai[1], Ichiro Kuwahira[3]

Department of Forensic Medicine, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, [1]Department of Cardiac Physiology, National Cerebral and Cardiovascular Center, Osaka, [2]Department of Forensic Medicine, Tokyo Medical University, [3]Department of Respiratory Medicine, Tokai University Tokyo Hospital, Tokyo, Japan

Abstract

Background and Aim: Animals that have been exposed to intermittent or chronic hypoxia (CH) for long periods are often used for basic research into various pulmonary and cardiac conditions. In standard hypoxia-inducing systems, gas cylinders are used to supply the hypoxic gas. Thus, it is necessary to exchange the cylinders very often.

Methods: To resolve this difficulty, we have developed a system including an N_2 gas generator and an air compressor that is capable of inducing intermittent or CH.

Results: The hypoxic system we have developed has the following three advantages: (1) It enables persistent exposure to stable levels of intermittent or CH, (2) both the level and duration of hypoxia can be controlled, and (3) it has low initial and running costs.

Conclusion: This novel system makes it easy to induce stable hypoxic conditions in the laboratory and has low initial and running costs.

Key words: Chronic hypoxia, hypoxia-inducing systems, intermittent hypoxia

Received: 20th November, 2014; ***Revised:*** 15th December, 2014; ***Accepted:*** 27th December, 2014

INTRODUCTION

Clinical and environmental conditions such as obstructive and central sleep apnea syndrome (SAS), bronchial asthma, and transient exposure to high altitudes are characterized by intermittent hypoxia (IH).[1] Patients with these conditions can exhibit normal arterial PO_2 (PaO_2) concentrations between episodes of hypoxia, which can vary in duration and intensity. It has been reported that SAS is a major independent risk factor for cardiovascular diseases such as systemic arterial hypertension, myocardial infarction, cerebrovascular dysfunction, and idiopathic sudden death.[2] In animals and humans, long or frequent hypoxic episodes and chronic hypoxia (CH) are also associated with cardiovascular changes such as persistent polycythemia, systemic and pulmonary arterial hypertension, and right ventricular hypertrophy. The mechanisms underlying the adverse consequences of IH and CH have been examined in several studies. In order to produce normobaric hypoxic conditions in the laboratory, a mixture of N_2 and O_2 gas is usually supplied from cylinders.[3] However, this causes various difficulties during animal experiments performed under hypoxia. For example, it is necessary to constantly check the amount of gas remaining in the cylinders. In addition, it is very hard to frequently exchange cylinders during experiments. For this reason, we tried to use an N_2 gas generator and an air compressor instead of gas cylinders to produce hypoxic gas for inducing IH or CH, for which the N_2 gas was obtained from the surrounding air.

Access this article online	
Quick Response Code:	Website: www.ijcep.org
	DOI: 10.4103/2348-8093.149770

MATERIALS AND METHODS

Figure 1 depicts a schematic representation of the newly developed system for inducing IH or CH. A pressure swing adsorption (PSA) type N_2 generator (ECONOX version 2.10, ECOTS, Osaka, Japan) was used. During the gas separation process, carbon molecular sieve

Address for correspondence: Dr. Hisashi Nagai, Department of Forensic Medicine, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo, Tokyo 113-8519, Japan. E-mail: nagai@legalmed.jp

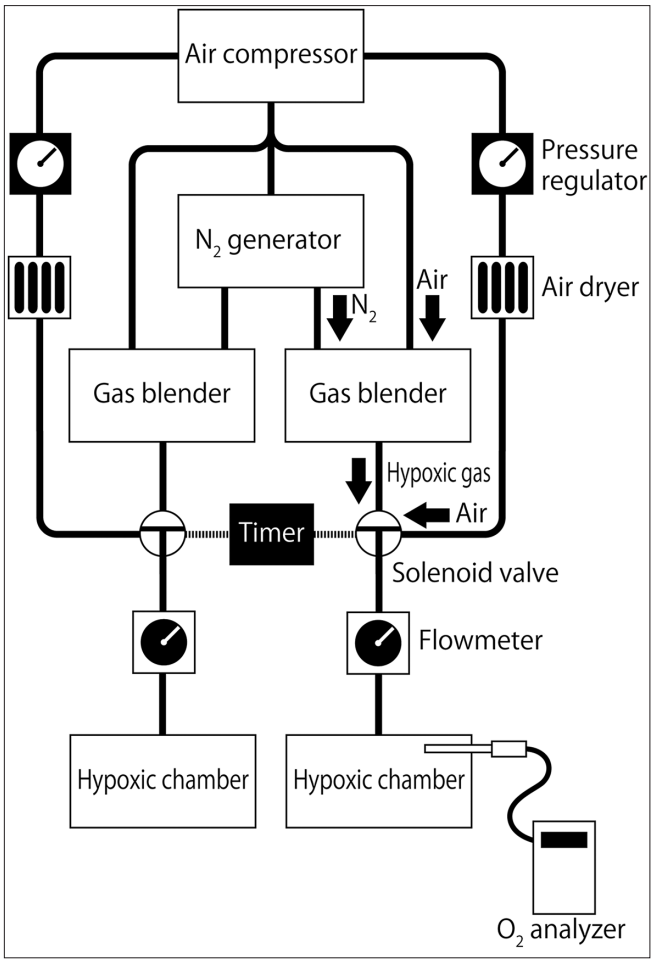

Figure 1: Experimental set-up. In order to induce intermittent hypoxia, hypoxic gas and compressed air are alternately delivered to two hypoxic chambers at the same time using a timed solenoid valve. The degree of hypoxia is controlled using a gas blender

preferentially adsorbed O_2 over N_2, which enabled the N_2 to pass through as a product gas at pressure. Such PSA technology is widely used for the recovery of high-purity N_2 (99.00–99.99% N_2 depending on gas flow) in industrial facilities. The generator can produce a maximum of 40 L N_2/min. An air compressor (oil-free scroll type, Smart Air SLP-15EBD, ANEST IWATA, Yokohama, Japan) was connected to the generator, and the maximum flow rate from the air compressor was 165 L/min. In order to induce IH, the hypoxic gas and compressed air were alternately delivered to two hypoxic chambers at the same time using a timed solenoid valve. The level of hypoxia was controlled using a gas blender. The flow rate of the hypoxic gas was regulated by a flow meter in the gas blender, and the flow rate of the compressed air was regulated by a pressure regulator. The flow rate to each chamber was continuously monitored using a flow monitor. In order to induce CH, a hypoxic gas mixture prepared from N_2 and air was continuously delivered to the chambers without using the timed solenoid valve, and the O_2 concentration in the chamber was monitored with an O_2 analyzer (JKO-25 oxygen monitor version 3, JIKCO, Tokyo, Japan).

In order to simulate SAS, we induced IH in rats as follows. The IH treatment consisted of alternating 90-s cycles of normoxia (21% O_2) and hypoxia (reaching 4% O_2 at the nadir). The gas flushing the chamber was automatically switched from compressed air to N_2 and back to compressed air. N_2 was delivered to a bespoke Plexiglas chamber (27 cm × 44 cm × 19 cm, model KYN-370, Bioresearch Center, Tokyo, Japan) at a rate of 14 L/min in each experiment. Compressed air was delivered at a rate of 54 L/min in each experiment.

We determined the changes in the arterial concentrations of blood gases during IH in three rats. A PE-50 catheter was inserted 20 mm into the left femoral artery under isoflurane anesthesia and was used to obtain blood samples. After surgery, each rat was transferred to accommodation box and allowed to fully recover from the anesthesia. A box containing a rat was then placed in one of the chambers, and room air was circulated through it. The measurements obtained under normoxia were carried out first, then IH was induced as described above. The measurements obtained under hypoxia were carried out 90 s after the composition of the gas had been changed. The gas mixture was then switched back to air, and measurements were carried out 90 s after the abrogation of the hypoxic conditions. The arterial concentration of blood gases in the 0.2-ml sample of arterial blood were measured with a pH/blood gas analyzer (Radiometer ABL 800 FLEX).

Statistical analysis of data

All statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA). The results of the blood gas analysis are presented as mean ± standard deviation (SD) values. The data analyses were performed using one-way ANOVA followed by Dunnett's multiple comparisons test for the normoxia versus hypoxia and the normoxia versus the return to normoxia comparisons. $P < 0.05$ were considered to indicate significance in all statistical analyses.

All experiments were approved by the institutional animal care and use committee of the University of Tokyo and conducted in accordance with the guidelines of the Physiological Society of Japan.

RESULTS

Simultaneous measurements of the O_2 concentrations of the two hypoxic chambers showed that the changes in the O_2 concentrations of the two chambers were identical. As shown in Figure 2, the same pattern of hypoxic exposure

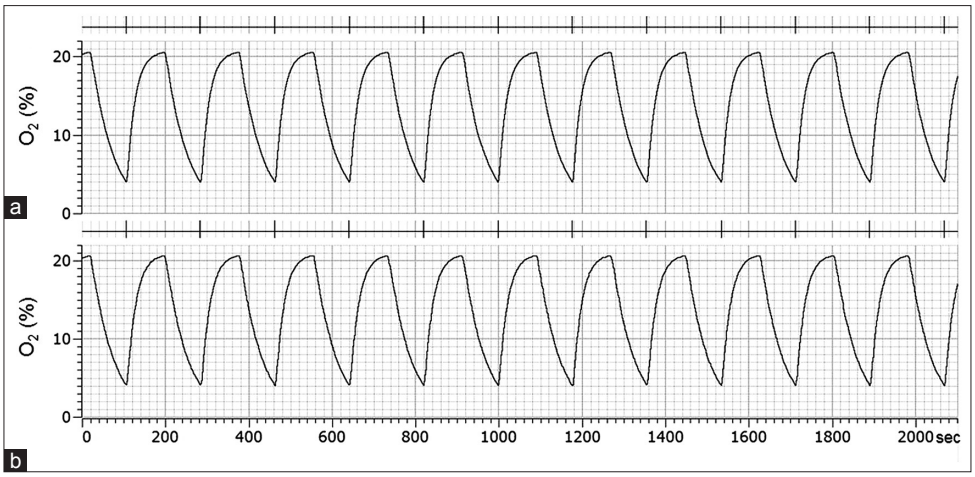

Figure 2: Changes in the O_2 concentrations of each chamber (a, b). Simultaneous measurements of the O_2 concentrations of the two hypoxic chambers showed that the changes in the O_2 concentrations of the two chambers were identical

followed by the normoxia was repeated in a sustainable manner throughout the experiment.

During hypoxia (fraction of inspired O_2: 4%), the rats' mean PaO_2, $PaCO_2$, and blood pH values were 25.4 ± 1.7 mmHg, 17.8 ± 0.7 mmHg, and 7.583 ± 0.020, respectively, and their mean blood $HCO3^-$ concentration was 16.4 ± 1.1 mEq/L. None of the rats developed syncope or died in spite of the severe hypoxemia induced. The rats' arterial blood gas concentrations normalized after the normoxic conditions were reinstated. As depicted in Table 1, the changes in the rats' arterial concentrations of blood gases exhibited reproducible patterns during the IH exposure experiments.

Table 1: Changes in arterial blood gas concentrations during normoxia and hypoxia

	Normoxia	Hypoxia	Return to Normoxia
pH	7.431±0.003	7.583±0.020**	7.410±0.017
PaO_2 (mmHg)	90.2±0.9	25.4±1.7**	83.3±8.6
$PaCO_2$ (mmHg)	34.5±1.0	17.8±0.7**	32.4±5.4
$HCO3^-$ (mEq/L)	22.1±0.7	16.4±1.1*	20.0±3.1

Arterial blood gas analysis detected severe hypoxemia and hypocapnia and acute respiratory alkalosis at an FiO_2 of 4% (n=3, mean±SD), *Significant difference compared with the normoxic conditions using one-way ANOVA. (*P<0.05, **P<0.01)

DISCUSSION

The three main advantages of our hypoxic exposure system are as follows: (1) It enables persistent exposure to stable levels of IH or CH, (2) both the level and duration of hypoxia can be controlled, and (3) it has low initial and running costs. The present hypoxic system includes a bespoke airtight animal cage, which is used as a hypoxic chamber. In our preliminary IH exposure experiments, the N_2 gas and air cylinders (both of which had capacities of 7000 L) became empty within 4 h. CH experiments also consume large amounts of hypoxic gas. Thus, it is necessary to frequently exchange gas cylinders during both IH and CH experiments. As the N_2 gas generator and air compressor continuously supply gas, our hypoxic system can expose experimental animals to stable levels of IH and CH for the desired period (including long durations), and it is not necessary to constantly check the amounts of gas remaining.

Using our hypoxic system, it is possible to set the duration of hypoxia and the O_2 concentration in the chamber during the hypoxic phase. In addition, the two hypoxic chambers are controlled by a single programmable timer. As depicted in Figure 1, we confirmed that the IH exposure conditions were identical in both chambers. Moreover, using this hypoxic system, we have previously found that prolonged periods of IH result in the attenuation of hypoxic pulmonary vasoconstriction.[4,5] In these reports, 7-week-old rats were exposed to IH (3-min periods of 4–21% O_2) for 8 h/day for 6 weeks. The duration of the hypoxic period remained constant, and the degree of hypoxia was maintained throughout the experiments.

In our experience, water tends to build up in the air compressor during the rainy season. Therefore, we recommend that high-performance dehumidifiers should be installed in the hypoxic system and laboratory to remove excess moisture. The initial cost of assembling the present hypoxic system, which includes the cost of the N_2 gas generator, air compressor, gas blender, and hypoxic chambers, was not very expensive (roughly 15,000 US dollars).

CONCLUSION

We have reported a new hypoxic exposure system involving an N_2 gas generator and an air compressor. This system makes it easy to induce stable hypoxic

conditions in the laboratory and has low initial and running costs.

ACKNOWLEDGEMENTS

We thank Ms. Yoko Takahari and Ms. Yoshiko Shinozaki for their expert technical assistance.

REFERENCES

1. Xi L, Serebrovskaya TV. Intermittent Hypoxia: From Molecular Mechanisms to Clinical Applications (Physiology-Laboratory and Clinical Research). New York: Nova Science Pub Inc.; 2010.
2. Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. Lancet 2009;373:82-93.
3. Dematteis M, Godin-Ribuot D, Arnaud C, Ribuot C, Stanke-Labesque F, Pépin JL, *et al.* Cardiovascular consequences of sleep-disordered breathing: Contribution of animal models to understanding the human disease. ILAR J 2009;50:262-81.
4. Nagai H, Kuwahira I, Schwenke DO, Tsuchimochi H, Nara A, Inagaki T, *et al.* ß2-Adrenergic receptor-dependent attenuation of hypoxic pulmonary vasoconstriction prevents progression of pulmonary arterial hypertension in intermittent hypoxic rats. PLoS One 2014;9:e110693.
5. Shirai M, Tsuchimochi H, Nagai H, Gray E, Pearson JT, Sonobe T, *et al.* Pulmonary vascular tone is dependent on the central modulation of sympathetic nerve activity following chronic intermittent hypoxia. Basic Res Cardiol 2014;109:432.

How to cite this article: Nagai H, Tsuchimochi H, Yoshida Ki, Shirai M, Kuwahira I. A novel system including an N 2 gas generator and an air compressor for inducing intermittent or chronic hypoxia. Int J Clin Exp Physiol 2014;1:307-10.

Source of Support: Nil, **Conflict of Interest:** Nil.