行政院國家科學委員會專題研究計畫 成果報告

活性氧族群造成大白鼠呼吸道過度反應的神經與介質機轉
(第 2 年)
研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 96-2628-B-040-020-MY2
執 行 期 間 ：97 年 08 月 01 日至 98 年 10 月 31 日
執 行 單 位 ：中山醫學大學醫學系生理學科

計 畫 主 持 人：阮婷
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報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 99 年 04 月 28 日

行政院國家科學委員會補助專題研究計畫 成果報告

活性氧族群引發大白鼠肺迷走 C 纖維感覺神經過度敏感的機制
Mechanisms Underlying Hypersensitivity of Lung Vagal C-fiber Afferents Induced by Reactive Oxygen Species in Rats

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計畫主持人： 阮婷
共同主持人： 高毓儒
計畫參與人員： 林鈺容

處理方式： 不公開

執行單位： 中山醫學大學生理科

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中文摘要

我們假設當大白鼠肺中的活性氧族群增加時會使得肺迷走C纖維變的感覺敏感而導致呼吸反射反應被擴大。為了證實此假說，我們讓動物自發性的吸入霧化的過氧化氫(H_2O_2)以增加動物肺中活性氧族群的量，並比較在吸入過氧化氫前後由靜脈注射辣椒素、phenylbiguanide或是ATP（此三種藥肺迷走C纖維常用的刺激物）所引發的呼吸反射反應的大小。我們以肺迷走C纖維被興奮所引起典型的反射反應－呼氣延長的時間(apnea)當做呼吸反射反應強度的指標。實驗中，我們發現隨著吸入的過氧化氫劑量愈高，由上述三種化學刺激物所引發的呼氣延長反應被增強的程度也愈高；而這樣經由肺中過氧化氫增加所增強的反射反應效用至少可以維持十分鐘，但在四十分鐘後則消失。此外，我們也觀察到，無論動物是否吸入過氧化氫，若先將迷走神經用辣椒素處理（可選擇性阻斷肺迷走 C 纖維的傳導）或切除二側迷走神經，再給予先前提到的三種刺激物，由其所引發的呼吸暫停反應會完全消失；假神經處理則不受影響。事先處理過氧化氫分解酶、氫氧自由基清除劑，再以phenylbiguanide為刺激物，我們發現由過氧化氫所增強的呼吸反射反應程度減少了，而前處理加熱去活化的過氧化氫分解酶、氫氧自由基清除劑溶劑增強的效用則不受影響。更進一步，我們觀察到，因過氧化氫所增大的呼吸反射反應會因事先給予TRPV1 受器拮抗劑、TRPA1 受器拮抗劑或是P2X受器拮抗劑而部分減弱，但不受前處理這三種受器拮抗劑的溶劑所影響。由以上這些結果，我們推測，大白鼠肺中的活性氧族群，特別是過氧化氫和氫氧自由基，會增強由肺迷走C纖維所引發的呼吸反射反應，而TRPV1 受器、TRPA1 受器和P2X受器也同時參與在其中。

關鍵詞：氧自由基、肺感覺敏感、TRPV1 受器、TRPA1 受器、P2X 受器

ABSTRACT

We tested the hypothesis that increased pulmonary reactive oxygen species (ROS) can sensitize lung vagal C-fiber afferents (LVCFAs) resulting in enhanced airway reflex reactivity in anesthetized rats. Pulmonary ROS was increased by delivery of 0.025 or 0.05 % aerosolized H_2O_2 for 90 s into the lungs while animals breathed spontaneously. Apneic responses to intravenous administration of capsaicin, α,β-methylene-ATP (α,β-meATP) and phenylbiguanide (3 stimulants for LVCFAs) before and after H_2O_2 inhalation were measured as the index of airway reflex reactivity. We found that the apneic responses to each of these chemical stimulants were augmented by H_2O_2 inhalation in a dose-dependent fashion. The augmented effect of H_2O_2 lasted for at least 10 min and vanished within 40 min. Perivagal capsaicin treatment (a procedure that selectively blocks LVCFAs) or vagotomy totally abolished the apneic responses to each of these chemical stimulants in animals with or without H_2O_2 inhalation, while sham vagi treatment failed to do so. The enhanced airway reflex reactivity to phenylbiguanide was reduced by pretreatment with dimethylthiourea [a hydroxyl radical scavenger] and was prevented by pretreatment with catalase (an enzyme catalyzing H_2O_2), but was unaffected by pretreatment with vehicle of dimethylthiourea or heat-inactivated catalase. Furthermore, this enhanced airway reflex reactivity was significantly attenuated by pretreatment with capsazepine (an antagonist of TRPV1 receptors), HC-030031 (an antagonist of TRPA1 receptors), or *iso*-PPADS (an antagonist of P2X receptors), but was no influenced by their vehicles. These results suggest that pulmonary ROS, especially H_2O_2 and hydroxyl radical, can augment LVCFAs-mediated apneic reflex in rats and that this enhanced airway reflex reactivity involves the function of TRPV1, TRPA1, and P2X receptors.

Keyword: oxygen radicals, lung sensory sensitization, TRPV1 receptors, TRPA1 receptors, P2X receptors

INDRODUCTION

Asthma is characterized by increased pulmonary ROS and airway hyperreactivity (AHR). LVCFAs, whose nerve endings possess TRPV1, TRPA1, and P2X receptors, have been implicated in the development of AHR and pathogenesis of asthma. The possibility that an increase in pulmonary ROS may sensitize LVCFAs resulting in enhanced airway reflex reactivity remains to be investigated.

OBJECTIVES

(1) To study whether an increase in pulmonary ROS by H_2O_2 inhalation may enhance the LVCFAs-mediated airway reflexes in rats.

(2) To exam the importance of ROS in this H_2O_2-induced AHR.

(3) To delineate the involvement of TRPV1, TRPA1, and P2X receptors in this H_2O_2-induced AHR.

METHODS

Pulmonary ROS was increased by delivery of 0.025 or 0.05 % aerosolized H_2O_2 for 90 s into the lungs of anesthetized male Sprague-Dawley rats while animals breathed spontaneously. Respiratory flow, tidal volume, and arterial blood pressure were continuously monitored. Apneic responses to intravenous administration of capsaicin, αβ-meATP and phenylbiguanide before and after H_2O_2 inhalation were measured as the index of airway reflex reactivity. Apneic Index (%) was defined as apneic duration/baseline expiratory time. Study was repeated after various experimental interventions including nerve treatments, ROS scavengers and antagonists of TRPV1, TRPA1, and P2X receptors.

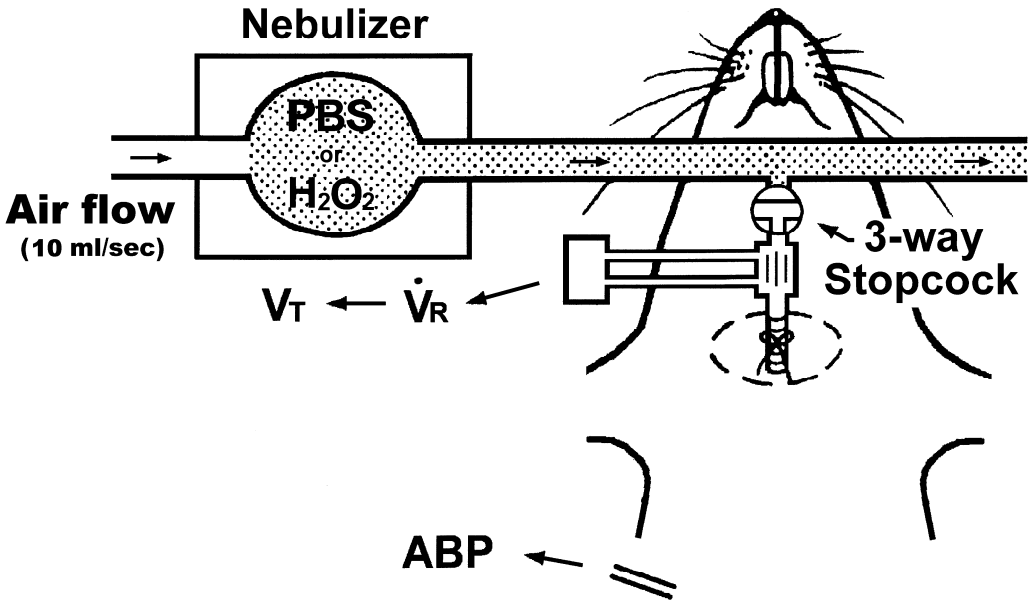

Figure 1. Schematic illustration showing the experimental setup. Animals breathed room air during control period. For H_2O_2 challenges, the 3-way stopcock was turned so that animals inhaled aerosolized H_2O_2 spontaneously via a sidearm from the lumen of the outlet tubing of the nebulizer. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure.

RESULTS

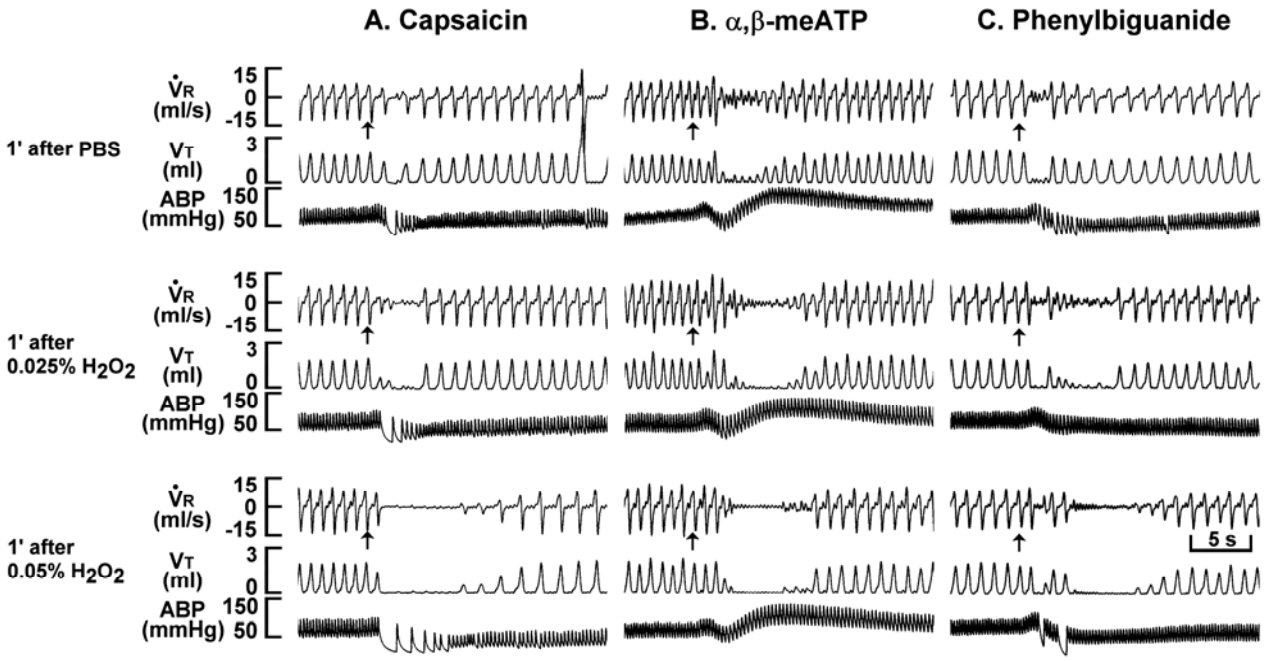

Figure 2. Apneic responses to intravenous injections of capsaicin (A), α,β-meATP (B), or phenylbiguanide (C) after inhalation of PBS, 0.025% and 0.05% H_2O_2 in three anesthetized rats. Capsaicin (0.5 μg/kg), αβ-meATP, (7.5 μg/kg) and phenylbiguanide (5 μg/kg) are a TRPV1 receptor agonist, P2X receptor agonist and serotonin 5-HT_3 receptor agonist, respectively. They are also chemical stimulants for LVCFAs. The onset of stimulant challenge is indicated by arrows. Between the inhalation of PBS and H_2O_2 or two H_2O_2 inhalations, 40 min were allowed to elapse. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure. Note that H_2O_2 dose-dependently augmented the apneic response to each stimulant.

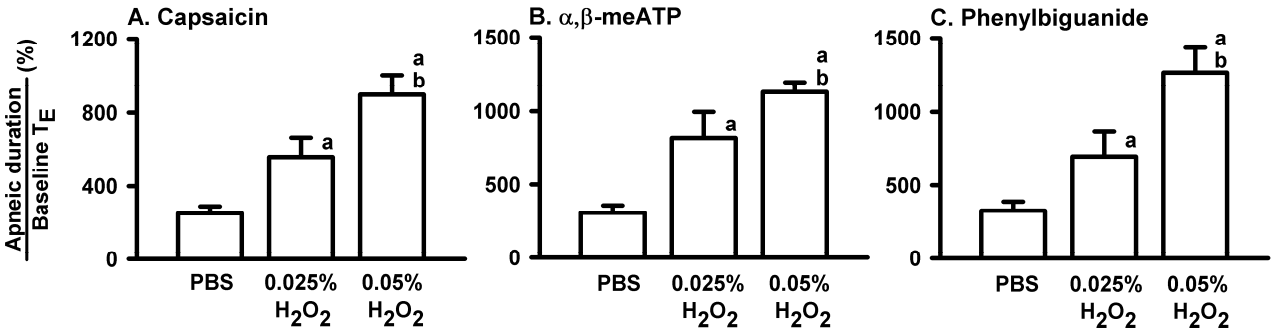

Figure 3. Augmentation of apneic responses to capsaicin (A), α,β-meATP (B), or phenylbiguanide (C) after inhalation of PBS, 0.025% and 0.05% H_2O_2 in three study groups. T_E, expiratory time; *, significantly different from baseline; a, significantly different from response after PBS; b, significantly different from response after 0.025% H_2O_2. Data in each group are mean ± SE from 8 rats. See legend of figure 2 for further explanation.

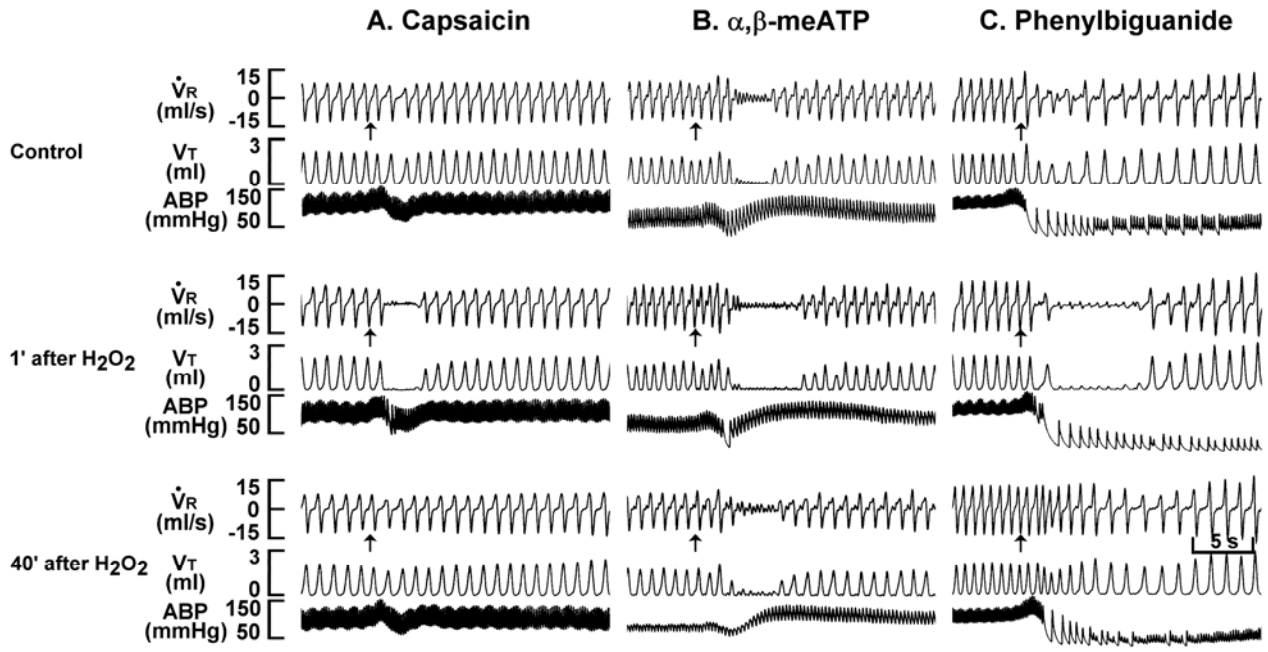

Figure 4. Apneic responses to intravenous injections of capsaicin (A), α,β-meATP (B), or phenylbiguanide (C) before and after one inhalation of 0.05 % H_2O_2 in three anesthetized rats. The onset of stimulant challenge is indicated by arrows. H_2O_2 inhalation was performed 40 min after obtaining the control response. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure. Note that the augmentation of the apneic response to stimulants by H_2O_2 occurred at 1 min after inhalation and vanished at 40 min after inhalation. See legend of figure 2 for further explanation.

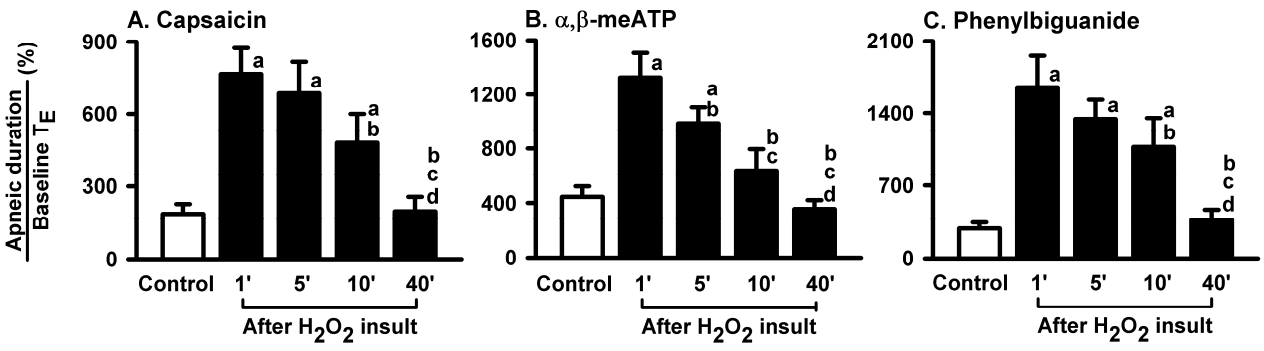

Figure 5. Time course of the augmentation of apneic responses to capsaicin (A), α,β-meATP (B), or phenylbiguanide (C) after one inhalation 0.05% H_2O_2 in three study groups. T_E, expiratory time; a, significantly different from the control response; b, significantly different from the response at 1 min after inhalation. c, significantly different from the response at 5 min after inhalation. d, significantly different from the response at 10 min after inhalation. Data in each group are mean ± SE from 8 rats. See legend of figure 2 for further explanation.

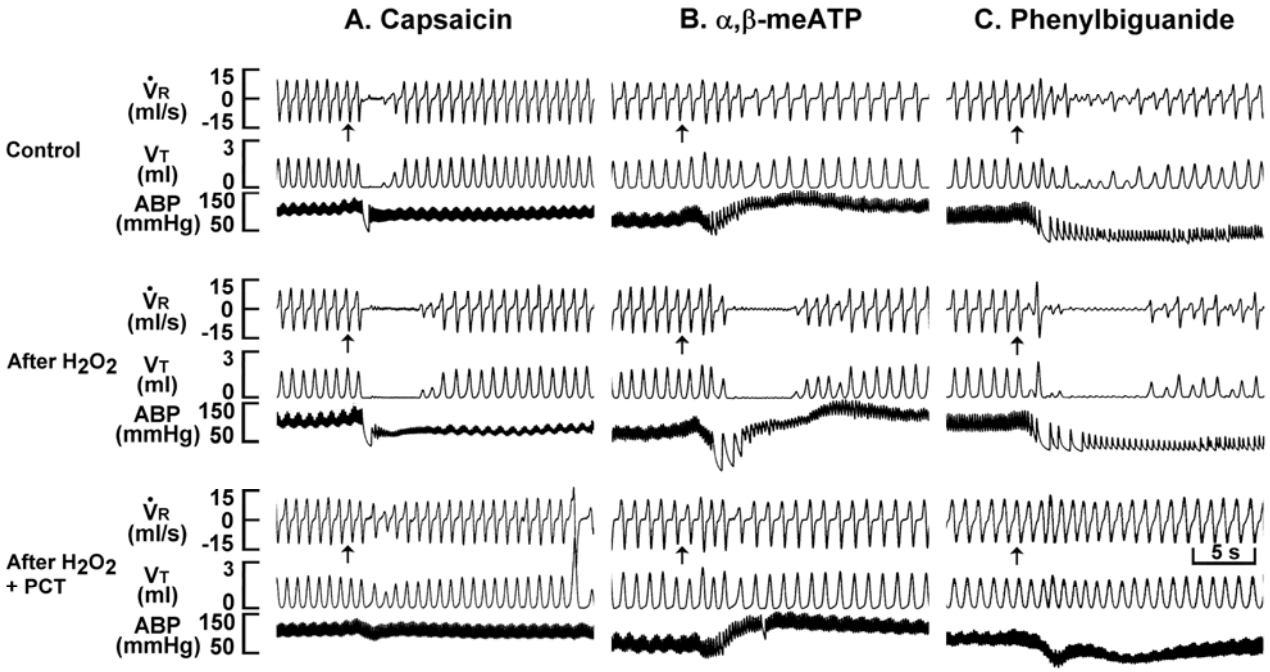

Figure 6. Apneic responses to intravenous injections of capsaicin (A), α,β-meATP (B), or phenylbiguanide (C) before and after two inhalations of 0.05 % H_2O_2 in three anesthetized rats. The first H_2O_2 inhalation was performed 40 min after obtaining the control response. The second H_2O_2 inhalation was performed 40 min after the first and after perivagal capsaicin treatment (H_2O_2 +PCT). Intravenous injections of these stimulants were performed 1 min after each H_2O_2 inhalation. The onset of stimulant challenge is indicated by arrows. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure. Note that the apneic responses to stimulants following the second H_2O_2 inhalation were abolished by PCT, suggesting that they were mediated through LVCFAs. See legend of figure 2 for further explanation.

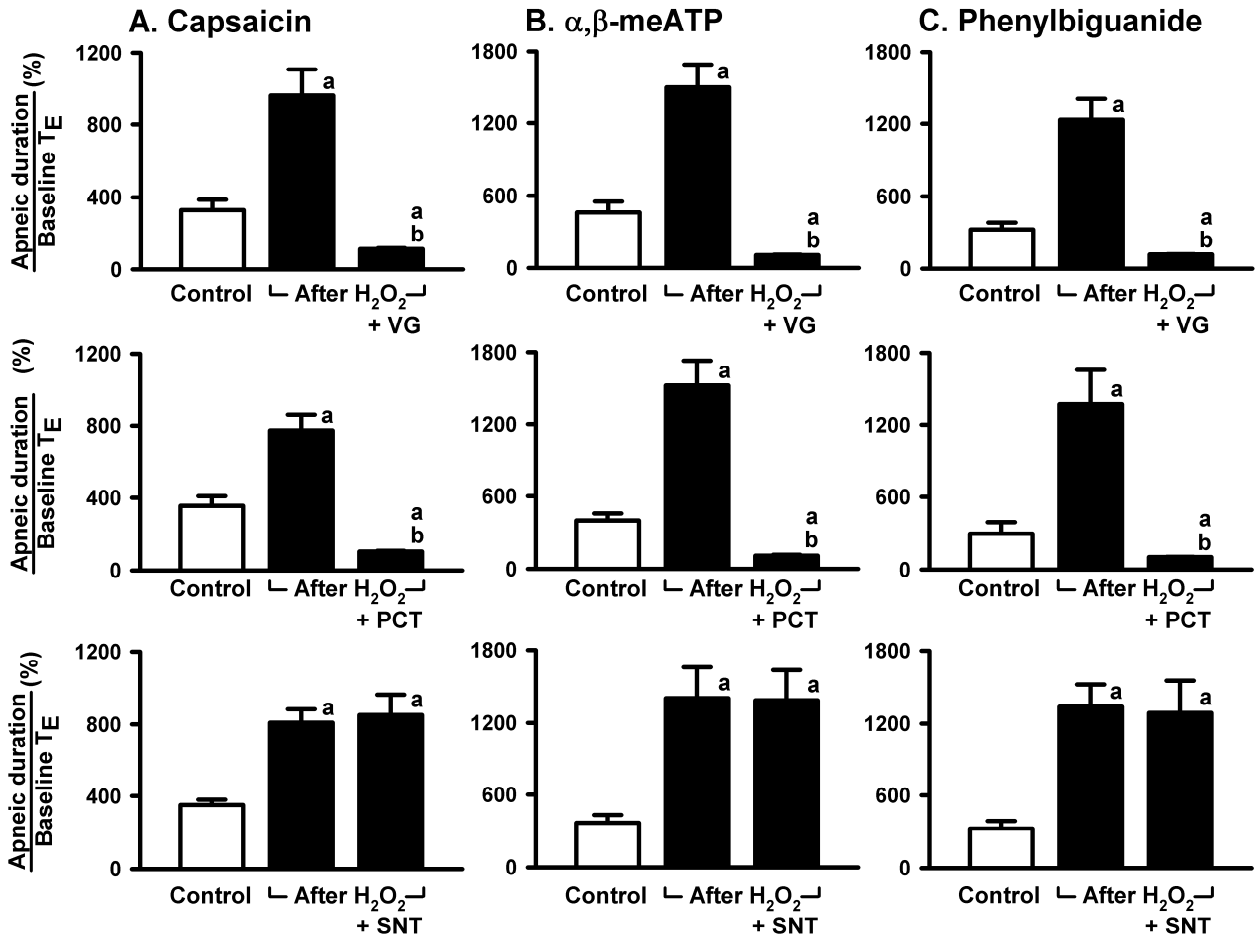

Figure 7. Effects of various vagus nerve treatments on the H_2O_2-induced augmentation of apneic responses to capsaicin (A), α,β-meATP (B), or phenylbiguanide (C) in nine study groups. VG, bilateral vagotmy; PCT, perivagal capsaicin treatment; SNT, sham nerve treatment; T_E, expiratory time; a, significantly different from the control response; b, significantly different from the response before nerve treatment. Data in each group are mean ± SE from 8 rats. See legend of figure 2 for further explanation.

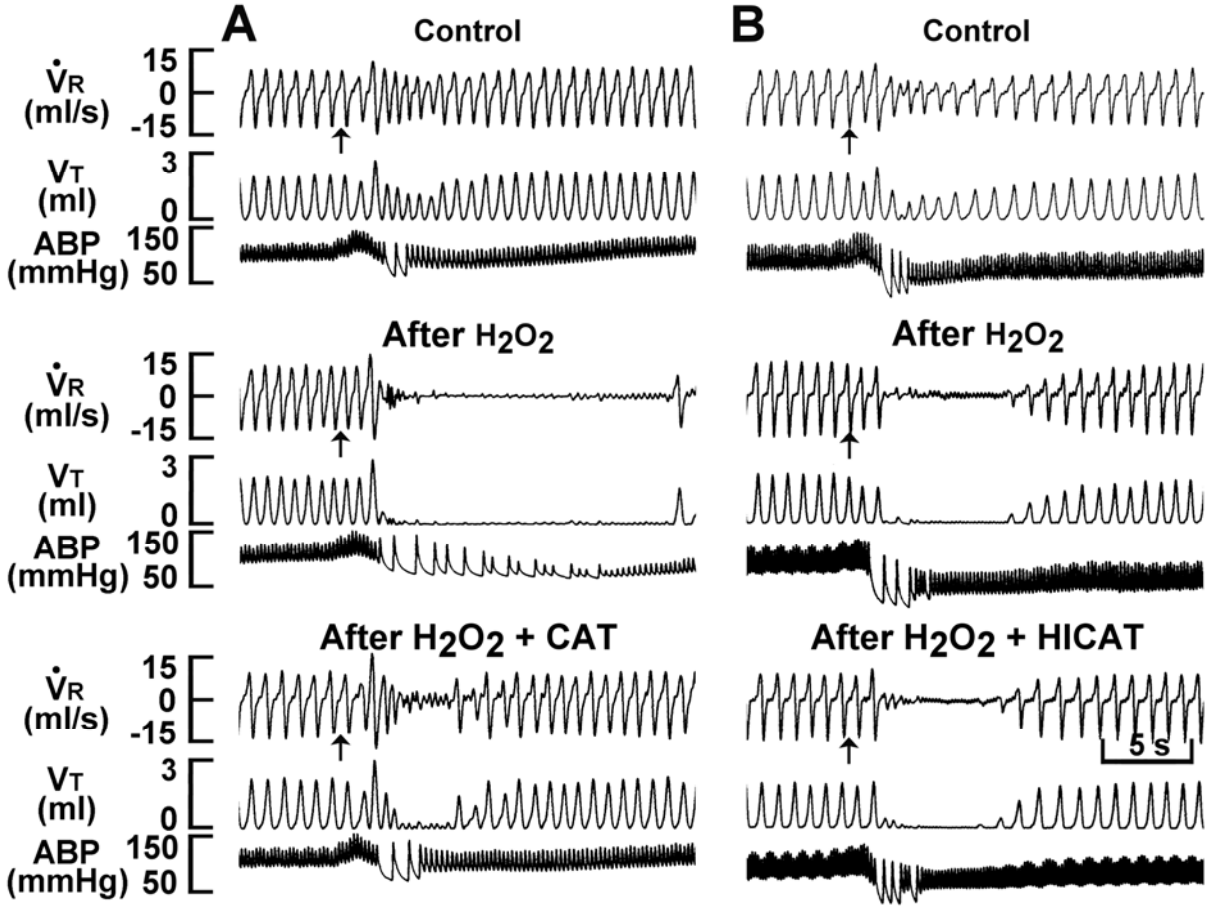

Figure 8. Apneic responses to intravenous injections of phenylbiguanide before and after two inhalations of 0.05 % H_2O_2 in two anesthetized rats. Phenylbiguanide (5 μg/kg) is a serotonin 5-HT_3 receptor agonist. The first H_2O_2 inhalation was performed 40 min after obtaining the control response. The second H_2O_2 inhalation was performed 40 min after the first and after pretreatment with catalase (H_2O_2 +CAT; A) or heat-inactivated catalase (H_2O_2 +HICAT; B). CAT (13500 IU/ml) is an enzyme catalyzing H_2O_2, whereas HICAT is the heat-inactivated enzyme. Intravenous injections of phenylbiguanide were performed 1 min after each H_2O_2 inhalation. The onset of stimulant challenge is indicated by arrows. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure. Note that pretreatment with CAT prevented the H_2O_2-induced augmentation of the apneic response to phenylbiguanide, whereas pretreatment with HICAT failed to do so.

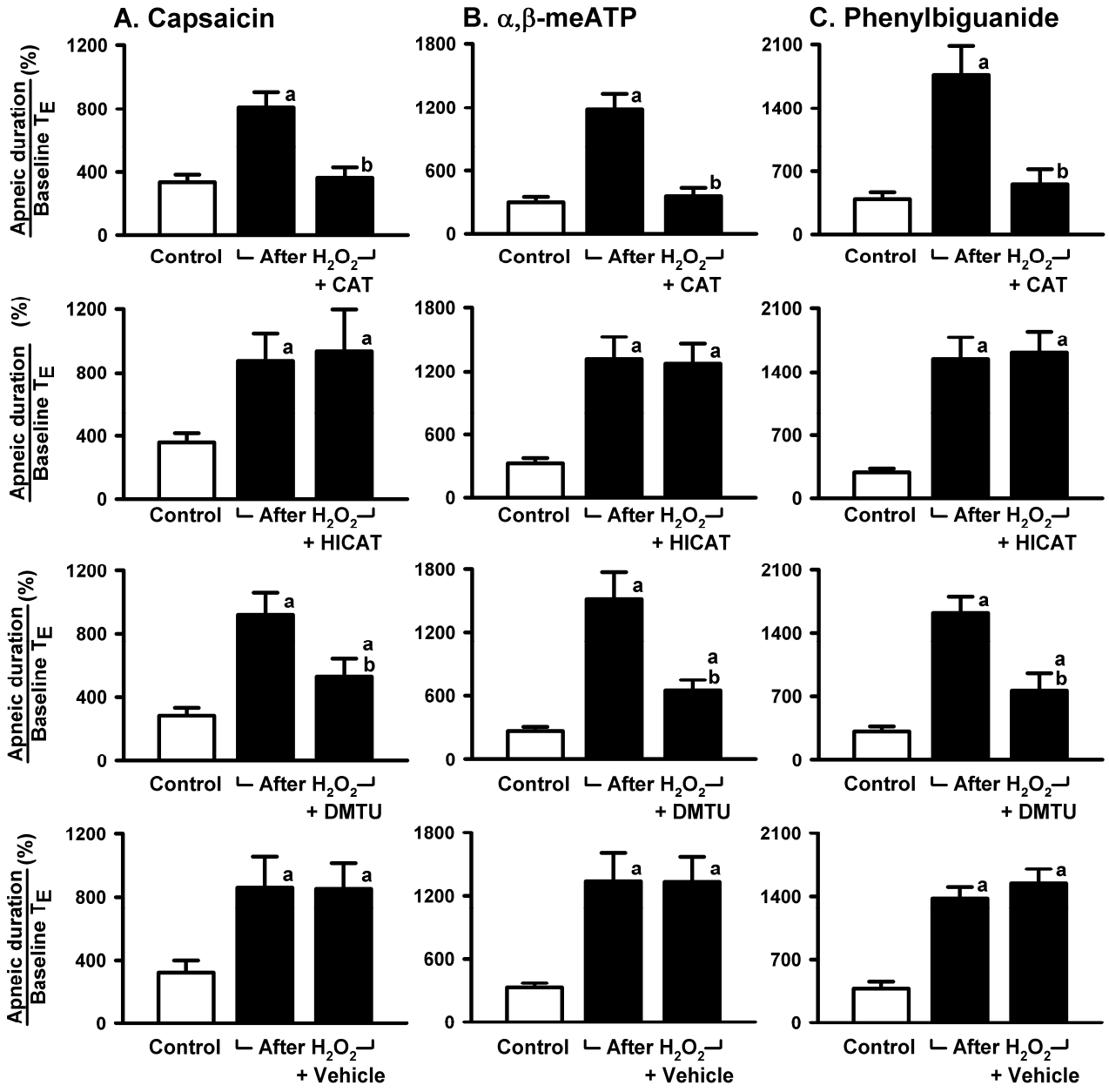

Figure 9. Effects of pretreatment with various pharmacological interventions on the H_2O_2-induced augmentation of apneic responses to phenylbiguanide in twelve study groups. CAT, catalase; HICAT, heat-inactivated catalase; DMTU, dimethylthiourea; vehicle, vehicle of DMTU. CAT (13500 IU/ml) is an enzyme catalyzing H_2O_2, whereas HICAT is the heat-inactivated enzyme. DMTU (1 g/kg) is a •OH scavenger. T_E, expiratory time; a, significantly different from the control response; b, significantly different from the response before pharmacological intervention. Data in each group are mean ± SE from 8 rats. See legend of figure 8 for further explanation.

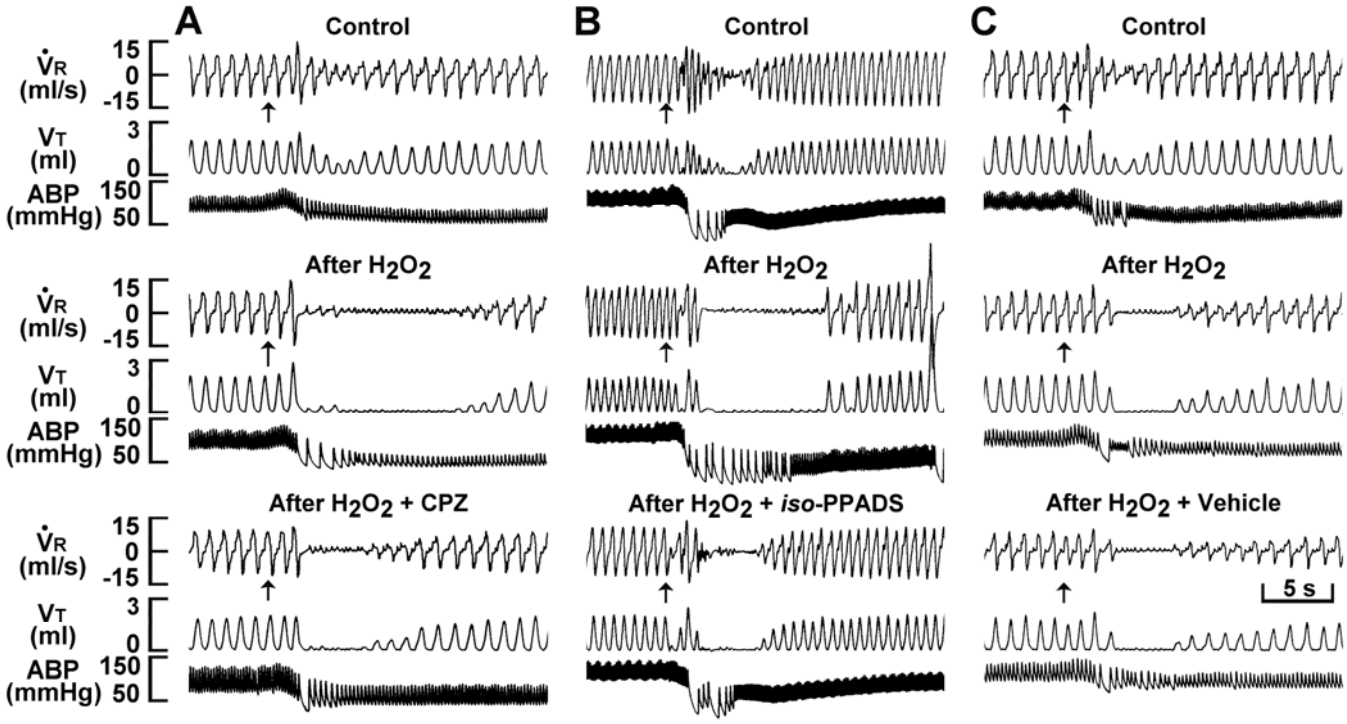

Figure 10. Apneic responses to intravenous injections of phenylbiguanide before and after two inhalations of 0.05 % H_2O_2 in three anesthetized rats. The first H_2O_2 inhalation was performed 40 min after obtaining the control response. The second H_2O_2 inhalation was performed 40 min after the first and after pretreatment with capsazepine (H_2O_2+CPZ; 3 mg/kg), *iso*-pyridoxalphosphate-6-azophenyl-2',5'-disulphonate (H_2O_2+*iso*-PPADS; 15 mg/kg)) or their vehicle (H_2O_2+Vehicle). CPZ is a TRPV1 receptor antagonist, whereas *iso*-PPADS is a P2X receptor antagonist. Intravenous injections of phenylbiguanide were performed 1 min after each H_2O_2 inhalation. The onset of stimulant challenge is indicated by arrows. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure. Note that pretreatment with CPZ or *iso*-PPADS suppressed the H_2O_2-induced augmentation of the apneic response to phenylbiguanide, whereas pretreatment with vehicle failed to do so. See legend of figure 8 for further explanation.

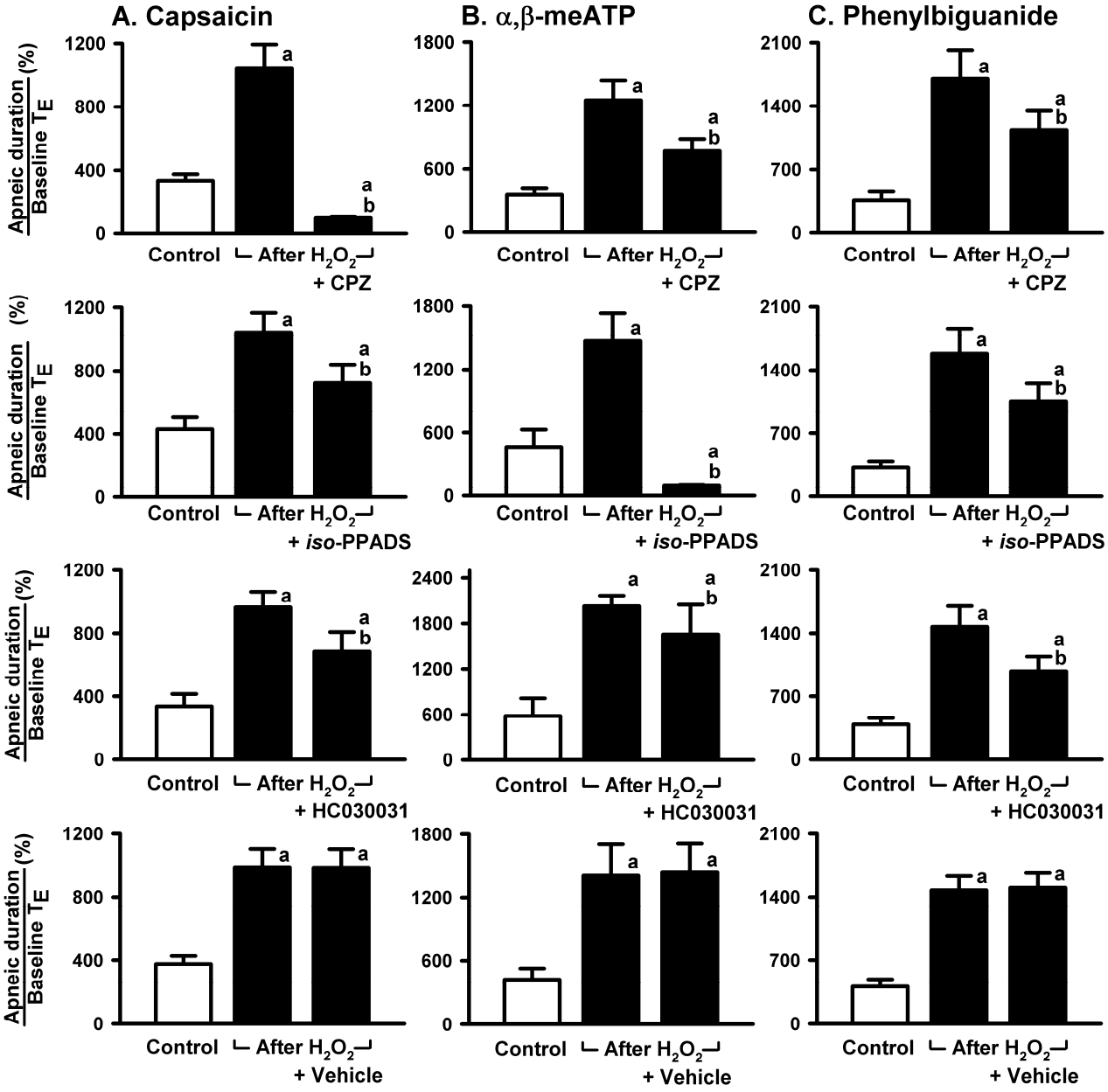

Figure 11. Effects of pretreatment with various pharmacological interventions on the H_2O_2-induced augmentation of apneic responses to phenylbiguanide in twelve study groups. CPZ, capsazepine; *iso*-PPADS, *iso*-pyridoxalphosphate-6-azophenyl-2',5'-disulphonate; vehicle, vehicle of CPZ, HC030032 and *iso*-PPADS; T_E, expiratory time; a, significantly different from the control response; b, significantly different from the response before intervention. Data in each group are mean ± SE from 8 rats. See legend of figure 8 for further explanation.

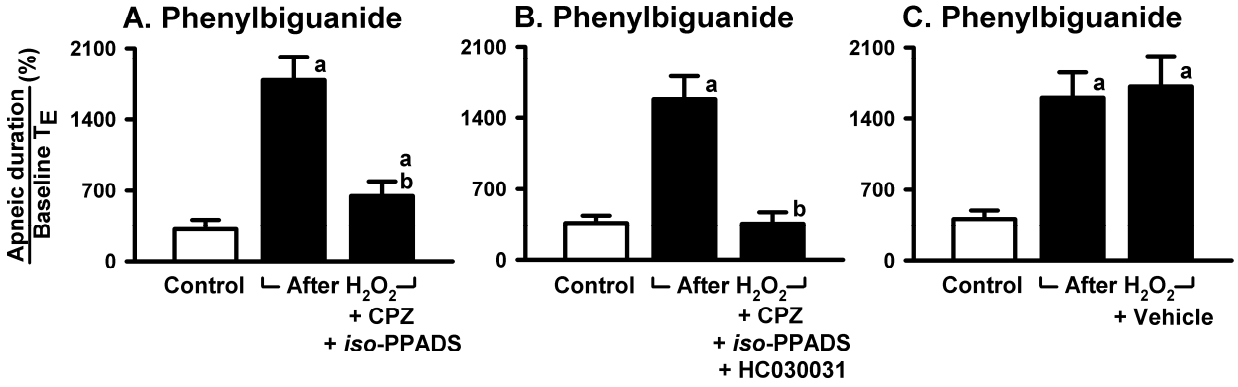

Figure 12. Effects of pretreatment with various pharmacological interventions on the H_2O_2-induced augmentation of apneic responses to phenylbiguanide in three study groups. CPZ, capsazepine; *iso*-PPADS, *iso*-pyridoxalphosphate-6-azophenyl-2',5'-disulphonate; vehicle, vehicle of CPZ and *iso*-PPADS; T_E, expiratory time; a, significantly different from the control response; b, significantly different from the response before intervention. Data in each group are mean ± SE from 8 rats. See legend of figure 8 for further explanation.

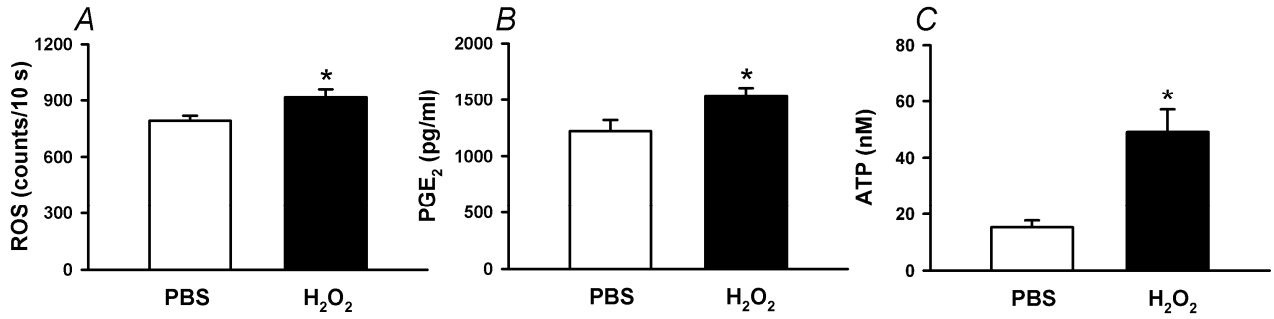

Figure 13.、動物吸入不同劑量H_2O_2肺泡灌洗液中ROS、PGE_2與ATP的含量平均圖。

圖A、B與C分別為吸入PBS (H_2O_2的溶劑；白色柱狀圖)與吸入 0.05%H_2O_2 (黑色柱狀圖)兩組動物，肺泡灌洗液中ROS、PGE_2與ATP的含量平均圖（每組n = 4~10）。實驗數據以平均值±標準差表示。＊：與吸入PBS組 有顯著差異。可以發現，吸入 0.05%H_2O_2的動物，其肺泡灌洗液中的ROS、PGE_2和ATP的含量顯著多於吸入PBS的動物。

Table 1. Average apneic response to an intravenous injection of capsaicin or lung inflation before and after various experimental interventions.

	Response to capsaicin (Apnoeic ratio, %)		Response to lung inflation (Apnoeic ratio, %)	
Intervention	Before	After	Before	After
VG	525 ± 87	110 ± 8*	2582 ± 372	106 ± 3*
PCT	623 ± 86	117 ± 8*	2362 ± 283	2284 ± 359
SNT	644 ± 98	689 ± 100	2679 ± 305	3022 ± 289

VG, bilateral vagotomy; PCT, perivagal capsaicin treatment; SNT, sham nerve treatment. *, significantly different from response before pretreatment. Data in each group are the mean ± SE from 8 rats. Note that PCT was selectively blocked the apneic response to capsaicin injection, VG was blocked both apneic response to capsaicin injection and to lung inflation, while SNT failed to do so.

Table 2. Average apneic response to intravenous injection of three receptor agonists before and after various antagonist pretreatments.

Group (n = 10)	Agonist	Response to Agonist (T_E, sec)			
		Before intervention		After intervention	
		Baseline	Peak response	Baseline	Peak response
CAT	Capsaicin	0.55 ± 0.07	3.26 ± 0.48*	0.53 ± 0.05	3.20 ± 0.55*
HICAT	Capsaicin	0.50 ± 0.04	3.65 ± 0.36*	0.49 ± 0.07	3.41 ± 0.41*
DMTU	Capsaicin	0.53 ± 0.04	4.08 ± 0.47*	0.59 ± 0.05	3.90 ± 0.55*
Vehicle-1	Capsaicin	0.52 ± 0.05	3.48 ± 0.85*	0.56 ± 0.06	3.61 ± 1.26*
CPZ	Capsaicin	0.49 ± 0.03	3.83 ± 0.79*	0.50 ± 0.07	0.56 ± 0.10
iso-PPADS	α,β-meATP	0.51 ± 0.07	6.18 ± 0.62*	0.57 ± 0.09	0.59 ± 0.09
CPZ+*iso*-PPADS	Phenylbiguanide	0.51 ± 0.05	7.01 ± 1.48*	0.50 ± 0.07	6.63 ± 1.22*
Vehicle-2	Phenylbiguanide	0.53 ± 0.05	5.90 ± 1.26*	0.57 ± 0.06	6.23 ± 1.10*
Trop	Phenylbiguanide	0.56 ± 0.04	7.94 ± 0.79*	0.57 ± 0.05	0.56 ± 0.05
HC030031	Capsaicin	0.48 ± 0.05	3.25 ± 0.67*	0.44 ± 0.04	3.41 ± 0.60*
Veh-3	Capsaicin	0.43 ± 0.05	3.15 ± 0.31*	0.41 ± 0.04	3.44 ± 0.36*

Capsaicin (1 μg/kg; a TRPV1 receptor agonist); αβ-meATP, α,β-methylene-ATP (10 μg/kg; a P2X receptor agonist); phenylbiguanide (6 μg/kg; a serotonin 5-HT_3 receptor agonist); CAT, catalase (13500 IU/ml; an enzyme catalyzing H_2O_2); HICAT, heat-inactivated catalase; DMTU, dimethylthiourea (1 g/kg; a ·OH scavenger); Vehicle-1, vehicle of dimethylthiourea; CPZ, capsazepine (3 mg/kg; a TRPV1 receptor antagonist); HC030031 (3 mg/kg; a TRPA1 receptor antagonist); *iso*-PPADS, *iso*-pyridoxalphosphate-6-azophenyl-2',5'-disulphonate (15 mg/kg; a P2X receptor antagonist); CPZ+*iso*-PPADS, a combination of CPZ and *iso*-PPADS; Vehicle-2, a combination of vehicles of CPZ and *iso*-PPADS; Vehicle-3, a combination of vehicles of CPZ, HC030031 and *iso*-PPADS; Trop, tropisetron (15 μg/kg; a serotonin 5-HT_3 receptor antagonist). T_E, expiratory time; *, significantly different from from corresponding baseline. Data in each group are the mean ± SE from 8 rats. Note that CPZ was selectively blocked the apneic response to capsaicin injection, *iso*-PPADS was selectively blocked the apneic response to α,β-meATP injection, while scavengers did not affect the apneic responses to capsaicin injections.

CONCLUSIONS

1. An increase in pulmonary ROS, especially H_2O_2 and ·OH, may augment LVCFA-mediated airway reflex in rats and that this enhanced airway reflex reactivity involves the function of TRPV1, TRPA1, and P2X receptors.
2. Therapeutic treatment of antioxidants and antagonists of TRPV1, TRPA1, or P2X receptors may potentially provide beneficial effects in alleviating airway hyperreactivity seen in asthmatic patients.

出席國際學術會議心得報告

計畫編號	NSC 96-2628-B-040 -020 -MY2
計畫名稱	活性氧族群造成大白鼠呼吸道過度反應的神經與介質機轉
出國人員姓名 服務機關及職稱	阮婷，中山醫學大學，生理科
會議時間地點	Kyoto, Japan, 07/27/2009 -08/01/2009
會議名稱	36th International Union of Physiological Sciences (IUPS2009)
發表論文題目	Augmentation of Vagal C-fiber Afferent-mediated Airway Reflex Reactivity by Pulmonary Reactive Oxygen Species in Anesthetized Rats

參加會議經過以及與會心得

第三十六屆國際生理科學會議（36th International Union of Physiological Sciences; IUPS 2009）於七月二十七日至八月一日在日本京都舉行。承蒙國科會補助經費使我能參加此一會議。此會議為一生理領域的國際科學會議，參與者來自世界各地的生理學家。與會期間，我還遇到許多在國內少有機會拜見的生理藥理教授帶著研究生一同參與此盛會。我於台北時間二十六日乘坐飛機離台，於當天下午飛抵日本大阪再轉乘巴士抵達京都。七月二十八日到八月一日有多場研討會。其中與呼吸生理相關的議程較為少。二十九日是我提報告的時段，與同領域的研究者交換了不少意見。由於飛機航班排程，我在八月一日下午搭機返國。筆者參加此次會議，除了學術上的交流外，也感覺到日本政府對於此種國際會議的重視，不但皇太子親自出席，日本也利用此機會於開幕式和晚宴的各項表演向國際介紹日本的文化與特色。