Effects of direct Cedrol inhalation into the lower airway on autonomic nervous activity in totally laryngectomized subjects

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Relationships between smell sensation and autonomic changes have been studied extensively.
- However, the possibility that odorants may also act on the lung and lower airway remains unknown.

WHAT THIS STUDY ADDS

- The present results provide the first evidence that the lung and lower airway exert an inhibitory influence on the cardiovascular system in response to Cedrol (odorant) in the air under physiological conditions.

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Keywords

blood pressure, heart rate, parasympathetic activity, spectral analysis, sympathetic activity, vagal nerve

Received

16 November 2006

Accepted

5 June 2007

Published OnlineEarly

22 October 2007

AIMS

Previous studies reported that Cedrol (odorant) inhalation (CI) induced changes in autonomic balance and baroreceptor sensitivity (BRS) in both healthy subjects and anosmic patients. This suggests that Cedrol may act on the lower airway, and that the pulmonary system may exert an inhibitory influence on the cardiovascular system.

METHOD

To test the above possibility, vaporized Cedrol ($64.0 \pm 7.7 \times 10^{-9}$ M) or blank air was directly inhaled through the lower airway from a hole in the trachea, but not through the upper airway, using totally laryngectomized subjects. During the experiment, ECG, systolic (SBP) and diastolic (DBP) blood pressures were measured. Sympathetic and parasympathetic nervous activity was estimated by spectral analyses of variability in these parameters (heart rate variability (HRV), SBP variability (SBPV) and DBP variability (DBPV)). BRS was computed from transfer gain between SBP and the R-R interval of the ECG.

RESULTS

SBP and DBP significantly decreased during CI, although there were no significant differences in HR and respiratory rate. BRS significantly increased during CI. The low frequency components of SBPV and DBPV (indices for sympathetic activity) significantly decreased during CI, while high frequency components of HRV (an index for parasympathetic activity) significantly increased.

CONCLUSIONS

The present experiment using totally laryngectomized patients replicated the similar results in healthy subjects who inhaled Cedrol through the nose, suppression of sympathetic outflow and increase in parasympathetic outflow. These results demonstrated that Cedrol acts on the lower airway and pulmonary system, and suggest a new target for drug therapy of hypertension.

Introduction

It is well known that odorants in the air have a powerful influence on behaviour and physiological functions in mammals, and the relationship between smell sensation and autonomic changes have been studied extensively. Some smells induced autonomic changes compatible with autonomic relaxation [1, 2], excitation [1, 3], and autonomic changes compatible with basic emotions [4]. Since the olfactory system has massive (direct or indirect) anatomical connections to the limbic system and hypothalamus [5], these neural circuits might provide a link between olfactory sensation and autonomic responses [6].

Cedrol is a crystalline natural substance derived from cedar wood (*Juniperus virginia*) oil. Cedrol or cedar wood oil, which diffuses a weak aroma, has been commonly used as an ingredient of shampoo, washing soap, facial soap, essence, etc. to enhance other fragrances. It has been reported that exposure to cedar wood oil increases Fos expression in the olfactory and the limbic system in rats [7, 8]. Furthermore, exposure to extracted cedar essence increased the amount of nonrapid eye movement (NREM) sleep in rats, decreased spontaneous activity and amount of wakefulness in rats, and decreased NREM sleep latency in humans [9], while exposure to cedar wood oil enhanced photic resetting of the circadian clock in rats [10]. These results suggest that some of the compounds in cedar wood essence such as Cedrol may cause sedation and suppression of sympathetic activity. Consistent with this suggestion, we have previously reported that Cedrol inhalation increased parasympathetic activity while it decreased sympathetic activity in healthy human subjects [6]. It has also induced an increase in baroreceptor sensitivity (BRS) in the same healthy human subjects [6].

However, the exact mechanisms of these effects remained to be elucidated since both centrally (olfactory)- and peripherally (nonolfactory)-mediated mechanisms were suggested [6]. That is, the human subjects inhaled Cedrol into the lower airway of the lung as well as the upper airway where the olfactory mucosa is located. It has been reported that stimulation of the bronchopulmonary C-fibre afferents in the lower airway by various chemical substances induced autonomic responses such as hypotension, bradycardia, apnoea, etc. (i.e. pulmonary chemoreflex) in animals [11, 12]. Furthermore, we reported a case report of an anosmic patient who showed a decrease in sympathetic activity and an increase in parasympathetic activity during Cedrol inhalation [13]. Consistent with these results, direct inhalation of Cedrol into the lower airway induced similar autonomic responses in rats [14]. These results suggest that Cedrol might act on the lower airway. In the present study, to test this possibility, Cedrol was inhaled directly into the lower airway using totally laryngectomized patients.

The effects of Cedrol on autonomic functions in these patients were investigated using noninvasive methods

with spectral data to evaluate peripheral autonomic nerve activity. Reliability of spectral data in assessing autonomic status has been reported; high frequency (HF) component of heart rate variability (HRV) reflects activity of vagal parasympathetic system [15], and its low frequency (LF) component includes both sympathetic and parasympathetic activities [16]. The LF/HF ratio of the HRV denotes the balance between sympathetic and parasympathetic nervous system [17]. LF power spectrum of blood pressure (BP) is an indirect measure of vasomotor sympathetic activity [18]. Baroreceptor sensitivity, which is associated with BP and parasympathetic activity, was estimated using spectral data of R-R interval of ECG and systolic BP [19]. Analyses by these noninvasive assessments of autonomic functions indicated similar effects of Cedrol on autonomic functions in totally laryngectomized patients to those in healthy subjects who inhaled Cedrol through the nose.

Methods

Subjects and materials

Eleven male subjects who had previously undergone total laryngectomy were studied (mean age, 66.8 ± 2.4 years). They were nonsmokers and none of them had any olfactory problems at the time of the experiment. All experimental procedures were carried out according to the ethics code of our institution with adequate understanding and consent of the subjects.

Subjects were asked to take adequate rest and sleep on the previous day, and to report to the laboratory at 08.00 h after fasting for 4 h before any form of physical exertion. Before the experiment their physical conditions were assessed and all subjects were suitable for the experiment.

Charcoal (Charcoal activated granular, Wako Pure Chemical Industries, Japan)-filtered room air was used as control air. For the test air, crystalline natural pure compound of Cedrol [3R-(3 α , 3a β , 6 α , 7 β , 8a α)]-octahydro-3, 6, 8, 8-tetramethyl-1H-3a, 7-methanoazulen-6-ol) (KAO Cosmetic Research Laboratory, Tokyo, Japan) was vaporized (see below in detail) and used.

Experimental setup and monitoring methods

The subjects were comfortably seated on an electrically driven reclining chair (chair with adjustments used for external dialysis) in a lit quiet $3 \times 3 \times 5$ m room with an exhaust fan to re-ventilate the room air continuously. The inclination of the upper body was set at 25° from the horizontal plane while the legs were kept in horizontal plane using the chair adjustments. A previous study reported that heart rate variability of subjects on the passive tilt less than 30° was similar to that in the supine position [20]. The left arm was kept at the level of the right atrium, and a noninvasive tonometric BP transducer, connected to a BP monitor (Jentow 7700, Colin, Japan),

Figure 1

A diagram of an experimental system using totally laryngectomized patients

was strapped over the left radial artery. The sphygmomanometer cuff of an oscillometric BP recording device for calibration of the tonometric transducer was attached to the upper left arm. Surface electrodes were attached to the chest for recording of the electrocardiogram (ECG). All of these data were digitized at 500Hz, displayed on a polygraph system, and stored on a hard disk (EEG-1100, Nihon Kohden, Japan).

Vaporized and blank air was presented using an olfactometer with separate Teflon-coated tubing and mask systems to prevent deposition of Cedrol particles. A mask was set up to cover the hole in the trachea in the anterior necks of the subjects. Charcoal-filtered room air was passed through a stainless steel container with a warmer to vaporize Cedrol at 90°C, and presented to the subjects in constant concentration ($14.2 \pm 1.7 \mu\text{g L}^{-1}$; $64.0 \pm 7.7 \cdot 10^{-9} \text{ M}$) at constant velocity (2.0 L min^{-1}) through the hole in the trachea (Figure 1). Blank air was similarly presented at 2.0 L min^{-1} except it did not pass through the stainless steel container. Output temperature of both odorized and blank air was monitored via an air flow thermo-sensor (KP 15097, Okazaki, Japan) at the end of the tubing near the mask, and was kept constant at $27.0 \pm 0.5^\circ\text{C}$.

Experimental procedure

The subjects were instructed to relax and breathe normally, and told that their autonomic functions would be measured during inhalation of vaporized or blank air. After plugging both the nostrils with cotton and setting up a

mask on the hole in the trachea, and various probes and electrodes on the subject, data were recorded for more than 30 min without any stimulus to check the monitoring devices and to accustom the subjects to the new environment. Then, to avoid possible prolonged effects of Cedrol, blank air was firstly presented for 10 min followed by vaporized air (Cedrol) for 10 min. The BP measurement was calibrated before each stimulus presentation. Our previous study, in which blank air was presented twice in the same way, indicated that there were no differences in autonomic functions between first and second exposure to blank air [6]. This experiment verified that the effects, if any, were due to Cedrol itself and not to relaxation over time. In the present study, this control experiment was omitted due to a specific condition of the patients (i.e. total laryngectomy). In the separate experiment, the subjects were tested in two different experimental protocols on 2 different experimental days, in which blank air was inhaled twice in the same time schedule, or blank air was inhaled firstly followed by Cedrol inhalation in the same time schedule, while BP was monitored. Comparison of BP between the second exposure to blank air and exposure to Cedrol indicated essentially the same results (data not shown). This confirmed that the effects of Cedrol were not due to relaxation over time.

Data analysis

Ten min recordings during presentation of blank and odorized air were taken for analysis. Beat to beat (R-R) intervals

of heart rate were measured by detecting a peak of QRS waves of the ECGs, and HR was estimated from the R-R intervals. Power spectral analyses of variability of these cardiovascular parameters [i.e. HR, systolic BP (SBP), diastolic BP (DBP)] were performed with fast Fourier transformation technique (FFT) (2048 points) through a Hamming window. Balance between sympathetic and parasympathetic outflow was estimated as a ratio of low (0.03–0.15 Hz) and high (0.15–0.5 Hz)-frequency components of the power spectrum of the HR variability (LFHRV/HFHRV), and parasympathetic outflow as the high-frequency component (HFHRV) [17, 19, 21]. LFHRV and HFHRV were then normalized by dividing by a total power (LFHRV + HFHRV) [21], and designated as nLFHRV and nHFHRV. We also estimated LF components derived from the power spectral analysis of the SBP and DBP variability (LFSBPV, LF DBPV) which reflected muscle sympathetic activity [22]. Furthermore, a ratio of LFSBPV to HFHRV (LFSBPV/HFHRV) was assessed as sympathovagal balance [6, 23].

Arterial baroreflex sensitivity (BRS) was estimated by spectral analysis using the 'α-index' method [18, 19, 24]. This sensitivity equals the gain (α-index) of the transfer function between the oscillations of SBP and HR in the LF range [25]. The BRS (α-index) was obtained from the formula:

$$\text{BRS} = [P_{\text{R-R(LF)}} / P_{\text{SBP(LF)}}]^{1/2}$$

where $P_{\text{R-R(LF)}}$ and $P_{\text{SBP(LF)}}$ represent low frequency components of the spectral power of the R-R interval (ECG) and of the SBP, respectively. The validity of this calculation requires that the squared-coherence between the two variability signals is greater than 0.5 [24].

To decrease the effects of physiological monitoring on respiratory functions, respiration rate (RR) was indirectly

measured. Since the respiratory system has an intimate relationship with the cardiovascular system, observed changes in autonomic parameters derived from HR and BP variability could be ascribed to changes in RR. In the present study, HR variability was firstly analyzed by the FFT, and then respiratory rate was estimated from a peak frequency in a high frequency band (0.15–0.5 Hz) of the resultant power spectrogram.

All data were expressed as mean $\pm$ SEM. Statistical analyses were performed by a paired *t*-test. A value of $P < 0.05$ was defined as statistically significant.

Results

Changes in peripheral physiological functions during Cedrol inhalation

Figure 2A shows examples of SBP changes during inhalation of blank air and Cedrol in three subjects (KG, MT, and SY). SBP was around 120–160 mmHg during exposure to blank air while SBP gradually decreased during Cedrol inhalation. However, HR was relatively constant compared with the changes in SBP (Figure 2B). Figure 3 shows the averaged BP (A), HR (B), and RR (C) of the 11 subjects during inhalation of blank air and Cedrol. The mean SBP and DBP significantly decreased during Cedrol inhalation (paired *t*-test, $P < 0.05$). In contrast, Cedrol inhalation did not change mean HR and RR (paired *t*-test, $P < 0.05$). To analyze quantitatively the effects of Cedrol on SBP, simple regression analysis of SBP before and during Cedrol inhalation was performed (Figure 4). Analysis of variance indicated that the linear regression line fit to the data was statistically significant ($F_{1,9} = 13.66$, $P < 0.01$). The regression line is $Y = 0.739X + 45.5$, where *Y* and *X* represent SBP during Cedrol and air inhalation, respectively. A large open circle

Figure 2

Examples of changes in SBP (A) and HR (B) before and after Cedrol inhalation in three subjects. Note that SBP in the three subjects decreased after Cedrol inhalation by 10–20 mmHg while the HR was relatively constant

Figure 3

Changes in mean BP (A), HR (B), and RR (C) after Cedrol inhalation. Note that mean SBP and DBP significantly decreased while HR and RR did not change. * $P < 0.05$ by paired t -test. Blank air, (□); Cedrol, (▨)

Figure 4

Correlation between SBP during air and Cedrol inhalation. Each filled circle indicates mean SBP during air and Cedrol inhalation in each subject in the present study. An open large circle indicates mean SBP during air and Cedrol inhalation in the young healthy subjects in the previous study [6]

in Figure 4 represents the mean SBP changes in healthy subjects [6], and is located within the 95% confidence limit. This suggests that the effects of Cedrol were similar across the subjects with different SBP. However, since the slope of the regression line for SBP during Cedrol inhalation plotted against SBP during air inhalation is lower than 1.0, the effect of Cedrol was greater on SBP in hypertensive than normotensive subjects.

Figure 5

Examples of the power spectra of SBPV during air (A) and Cedrol inhalation (B). Power spectra data of SBPV are derived from the data shown in Figure 2 (Subject KG). Note that the LF component of SBPV decreased during Cedrol inhalation

Changes in autonomic nervous functions during Cedrol inhalation

Figure 5 shows an example of spectral analysis of the SBP variability during inhalation of blank air (A) and Cedrol (B). The LF component of SBP variability decreased from 0.428 to 0.278 mmHg² after Cedrol inhalation. This suggested suppression of sympathetic activity during Cedrol inhalation, consistent with BP reduction by Cedrol. The statistical analyses indicated that the mean LF components of SBP and DBP variability (LFSBPV, LFDBPV) significantly decreased during Cedrol inhalation (paired t -test, $P < 0.05$) (Figures 6A, B). The following analyses of HR variability also support this conclusion. The mean HF component of HR variability (HFHRV) significantly increased during Cedrol inhalation (paired t -test, $P \leq 0.05$) (Figure 7A). Although Cedrol inhalation did not affect mean LF/HF ratio derived from HR variability (LFHRV/HFHRV) (paired t -test, $P \geq 0.05$) (data not shown), LFSBPV/HFHRV significantly decreased after Cedrol inhalation (paired t -test, $P \leq 0.01$) (Figure 7B). Furthermore, the mean normalized HFHRV (nHFHRV) significantly increased, while the mean normalized LFHRV (nLFHRV) significantly decreased during Cedrol inhalation (paired t -test, $P \leq 0.05$) (Figures 7C, D).

Figure 6

Changes in mean LF components of SBPV and DBPV (LFSBPV, A; LFD BPV, B) during Cedrol inhalation. Note that mean LFSBPV and LFD BPV significantly decreased during Cedrol inhalation. * $P < 0.05$ by paired t -test

Figure 7

Changes in the mean HF component of HRV (HFHRV, A), mean ratio of LF component of the SBPV to HF component of HRV (LFSBPV : HFHRV; B), mean normalized HFHRV (nHFHRV, C), and mean normalized LFHRV (nLFHRV, D) during Cedrol inhalation. Note that mean HFHRV and mean nHFHRV significantly increased, while LFSBPV : HFHRV and nLFHRV significantly decreased during Cedrol inhalation. * $P \leq 0.05$ by paired t -test

Results in Figure 8 show the statistical comparison of ratios between LF components of HR and SBP variability (α index), which reflects BRS. Since all data from the 11 subjects showed a squared coherence of more than 0.5, all these data were included in the analysis. The statistical

Figure 8

Changes in baroreceptor sensitivity (BRS) after Cedrol inhalation. Note that mean BRS significantly increased during Cedrol inhalation. * $P < 0.05$ by paired t -test

analysis indicated that baroreceptor sensitivity significantly increased during Cedrol inhalation (paired t -test, $P < 0.05$).

Discussion

Effects of Cedrol on autonomic functions in totally laryngectomized patients

The present results indicated that Cedrol inhalation induced SBP and DBP reduction while HR and RR did not change. Consistent with BP reduction, spectral analysis indicated that the LF component of SBP and DBP variability (LFSBPV, LFD BPV) significantly decreased during exposure to Cedrol. Furthermore, the HF component of HR variability (HFHRV) significantly increased, while the LFSBPV/HFHRV ratio reduced during inhalation of Cedrol. These changes in the cardiovascular parameters were consistent in terms of peripheral autonomic nerve activity, suggesting that exposure to Cedrol increased parasympathetic activity and reduced sympathetic activity.

Baroreceptor sensitivity significantly increased during exposure to Cedrol. Consistent with the present results, increase in baroreceptor sensitivity was associated with increase in parasympathetic activity (HFHRV) and reduction in sympathetic activity (LFSBPV, LFD BPV, LFSBPV/HFHRV) [26], and a reduction in BP [27]. It has been reported that baroreceptor sensitivity decreased with increasing mental arousal [27], physical exercise [28], mental stimulation [29], and emotional behaviour [30]. These results, along with lesion studies [31, 32], suggest that the higher brain areas in the forebrain such as the prefrontal cortex, hypothalamus, and limbic system modulate baroreflex. Afferent fibres from the baroreceptors

project to the medulla, pons, and hypothalamus [33] where the baroreflex might interact with the higher brain areas. Since Cedrol might activate the limbic system through the vagal nerve (see below), Cedrol might also release the baroreflex from the central inhibition through the limbic system.

Thus, the data in the present study replicated the similar results obtained in healthy subjects in our previous study [6], suppression of sympathetic outflow and increase in parasympathetic outflow. However, respiration rate did not change in the present study although this parameter decreased in the healthy subjects [6]. These differences might be attributed to differences of the subjects and/or differences in inhalation routes between the two studies. Further studies are required to elucidate the effects of Cedrol on the cardiovascular system; it would be interesting to assess cardiovascular changes during exposure of Cedrol only to the upper airway using the same laryngectomized subjects.

Possible mechanisms for the action of Cedrol

The present results suggest that Cedrol acts on the lower airway and pulmonary system, presumably on the peripheral nervous system in these regions. Since Cedrol inhalation did not modulate respiration rate (RR) in the present study, bronchopulmonary chemical receptors rather than lung stretch receptors plays an important role in these autonomic responses.

It has been reported that rats exposed to essential oil vapours displayed motility increment or decrement according to the type of the smells, and fragrance compounds were found in their serum [34]. These results suggest that Cedrol might directly act on the peripheral autonomic systems via a blood-borne route. For example, pulmonary vagal C-fibre (type J) receptors are implicated in autonomic responses to chemical compounds in the pulmonary circulation [35]. It is also possible that Cedrol directly acts on peripheral afferent fibres of the vagal nerve innervating the respiratory system through the epithelium. Our preliminary study using gas chromatography indicated that Cedrol was not detected in the blood after inhalation in rats (unpublished data). These results strongly suggest that Cedrol directly stimulates bronchopulmonary chemical receptors but not via the blood. Consistently, anatomical studies reported the existence of nerve endings of pulmonary vagal C-fibres in the airway mucosa [36, 37] and neuroepithelial bodies innervated by vagal sensory afferents which could be directly stimulated by inhaled air [38, 39]. These afferent fibres might detect chemical compounds in inhaled air to induce various autonomic responses [12].

The action of Cedrol in the lower airway

It has been reported that two types of the vagal sensory receptors, pulmonary unmyelinated C-fibre endings and rapidly adapting receptors (RARs), exhibit chemical sensi-

tivity [35, 39]. Especially, pulmonary C-fibres comprise about 75% of the vagal afferent fibres in the respiratory system [35]. Stimulation of these fibers elicited various pulmonary chemoreflexes such as hypotension, bradycardia, apnoea, cough, mucus secretion, etc. [40, 41]. However, the present results are distinct from these previous results since Cedrol inhalation specifically altered blood pressure, but not heart rate or respiratory movements.

Consistent with the present results, it is reported that vagal C-fibres were the dominant mediators of the tonic vasomotor inhibition exerted by cardiopulmonary receptors [42]. It is reported that selective chemical stimulation of pulmonary C-fibres produced cardiovascular depression, but not after lung denervation [11], while bronchial C-fibre stimulation did not produce such cardiovascular responses [43]. These results suggest that pulmonary C-fibres play an important role in the present results. Furthermore, a recent study using anaesthetized rats reported that direct Cedrol inhalation from the trachea activated pulmonary C-fibres, and changed autonomic functions [14]. These autonomic changes were abolished after vagotomy. It is noted that the previous studies reported genetic and functional diversity of bronchopulmonary C-fibre receptors [44, 45]. These results suggest that Cedrol stimulates a new type of receptor in the pulmonary C-fibres that selectively inhibits vasomotor tone through the central nervous system (see below in detail).

Neurophysiological studies reported that neurones in the nucleus tractus solitarius (NTS) responded to electrical and local chemical stimulation of pulmonary C-fibres [46, 47], and stimulation of the NTS neurones induced similar autonomic responses to peripheral chemical stimulation [48]. These results suggest that the NTS is a key structure for mediation of activity of pulmonary C-fibres. Furthermore, the NTS projects not only to the areas in the brainstem involved in autonomic control but also to the hypothalamus and the limbic system through the parabrachial nucleus [49, 50]. Non-invasive studies using humans and animals indicated that vagal stimulation induced alteration of brain activity in the olfactory bulb, brainstem, hypothalamus, and limbic system [51, 52]. Consistently, animal studies indicated that presentation of Cedar wood oil induced an increase in activity in the various brain areas in the olfactory and limbic systems [7, 8]. These areas send descending visceromotor outputs, and play an important role in autonomic functions [5]. Cedrol might induce autonomic alteration through these areas in the central nervous system. Further studies are necessary to elucidate whether a local lower brainstem- or forebrain-mediated pathway, or both the pathways are involved in the autonomic changes observed in the present study.

In conclusion, to test possibility that Cedrol acts on bronchopulmonary C-fibre afferents, autonomic functions were analyzed using totally laryngectomized patients while they inhaled Cedrol directly through the lower airway. The results indicated that during Cedrol inhalation,

SBP and DBP significantly decreased, and LF components of SBPV and DBPV also decreased. Consistently, the HF component of HRV and baroreceptor sensitivity (BRS) significantly increased. Thus, the data replicated the similar results found in the healthy subjects, suppression of sympathetic outflow and increase in parasympathetic outflow. These results suggest that Cedrol acts on the peripheral nervous system innervating the lower airway and pulmonary system. These results further suggest a new target for drug therapy for essential hypertension and sleep apnoea, in which elevated sympathetic activity contributes importantly to disease pathology [53].

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