Changes in respiratory sensations induced by lobeline after human bilateral lung transplantation

J. E. Butler*, A. Anand†, M. R. Crawford‡, A. R. Glanville§, D. K. McKenzie*‖, A. S. Paintal†, J. L. Taylor* and S. C. Gandevia*

**Prince of Wales Medical Research Institute, University of New South Wales, Sydney, ‖Departments of Respiratory Medicine and ‡Anaesthesia and Intensive Care, Prince of Wales Hospital, Sydney, §Heart and Lung Transplant Unit, St Vincent's Hospital, Sydney, Australia and †Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India*

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1. The sensations evoked by the injection of lobeline into the right antecubital vein were studied in 8 subjects after bilateral lung transplantation and 10 control subjects. In control subjects, two distinct sensations were experienced. There was an early noxious sensation (onset ~10 s) followed by a late sensation of breathlessness (onset ~26 s) associated with involuntary hyperventilation. The early sensation was accompanied by respiratory and cardiovascular changes.

2. In contrast to control subjects, the early respiratory events and the noxious sensations evoked by injections of lobeline (18–60 μg kg^{-1}) did not occur in subjects with recent bilateral lung transplantation. This suggests that the early respiratory sensations are mediated by the discharge of receptors in the lungs.

3. The late hyperventilation and the accompanying sensation of breathlessness occurred in both transplant and control subjects and are therefore likely to be mediated by receptors elsewhere in the body, presumably systemic arterial chemoreceptors stimulated by lobeline.

4. In control subjects, but not transplant subjects, there was a consistent decrease in mean arterial pressure associated with the lobeline injection. This suggests that pulmonary afferents mediate the hypotension.

5. For transplant subjects studied more than a year after transplantation, there was some evidence that the noxious respiratory sensations evoked by lobeline had returned. This suggests that some functional reinnervation of pulmonary afferents may occur.

In animals, the stimulation of pulmonary C fibres by the injection of phenyl diguanide causes potent cardiovascular and respiratory reflex effects such as hypotension, bradycardia and apnoea followed by hyperventilation as well as inhibitory viscerosomatic reflex effects on limb motoneurones. These reflex changes occur in many species including the cat whether it is decerebrate, anaesthetised, or awake (Ginzel & Eldred, 1969, 1970; Paintal, 1969, 1970, 1973; Deshpande & Devanandan, 1970; Schiemann & Schomburg, 1972; Kalia, 1973; Kalia *et al.* 1973; Ginzel, 1973; Anand & Paintal, 1980; Pickar *et al.* 1993).

In human subjects, the activation of these receptors by intravenous or right atrial injections is associated with a noxious sensation of respiratory discomfort in the throat and chest (Bevan & Murray, 1963; Jain *et al.* 1972; Raj *et al.* 1995; Gandevia *et al.* 1998*b*; for review see Paintal, 1986, 1995). The duration of the sensation is dose dependent but, in conscious human subjects, there was no evidence for the viscerosomatic reflex inhibition of limb motoneurones which is present in animals (Gandevia *et al.* 1998*b*). In the study by Gandevia *et al.* (1998*b*), two distinct types of sensations were reported, the early noxious sensation and the subsequent sensation of 'breathlessness' or 'air hunger'. It was assumed that the time course of the noxious sensations evoked by the injection of lobeline indicated the time course of pulmonary C fibre stimulation. Doses of lobeline between 40 and 70 μg (kg body weight)$^{-1}$ gave sensations lasting about 30 s.

The relatively short latency for the early sensation (~10 s in response to lobeline delivered to the right antecubital vein) does suggest that the noxious sensations are initiated by the activation of pulmonary afferents rather than receptors in the systemic circulation (Jain *et al.*

1972; Raj *et al.* 1995; Gandevia *et al.* 1998*b*). However, the duration of the noxious sensations at the doses used in our study (~30 s) was sufficiently long for systemic circulation of the lobeline. Therefore, to determine whether the extended noxious sensations associated with these injections of lobeline were evoked by non-pulmonary afferents, we studied subjects with complete pulmonary denervation.

The respiratory sensations along with the cardiovascular and respiratory changes associated with intravenous injections of lobeline were assessed in control subjects and in subjects after bilateral lung transplantation. We compared three aspects of the responses to lobeline in the control and transplant subjects: (i) the early noxious sensation and associated respiratory events such as coughing and apnoea, (ii) the later sensation of 'breathlessness' and the associated hyperventilation, and (iii) changes in mean arterial pressure and heart rate. This allowed us to examine which of the responses to lobeline injection can be attributed to pulmonary afferents and which are mediated by stimulation of other receptors. Some of the results have been presented in an abstract (Gandevia *et al.* 1998*a*).

METHODS

Studies were conducted on 8 subjects (5 males and 3 females, aged 20–52 years) who had received bilateral lung transplants (4 of these had undergone bilateral lung and heart transplantation), and 2 subjects who had undergone heart transplantation alone (2 males, aged 56 and 65 years). The majority (7 out of 8) of these subjects had been transplant recipients in the previous 13 months or less so the chance of reinnervation of the lungs was considered to be small (Higenbottam *et al.* 1989; Hathaway *et al.* 1993; Seals *et al.* 1993; Iber *et al.* 1995; Ramaekers *et al.* 1996). All transplant subjects were clinically well at the time of the study but were on a regimen of immunosuppression and antimicrobial medications. For the heart and lung recipients ($n = 4$) the trachea was divided 1 cm above the carina. For the bilateral lung recipients ($n = 4$), the main bronchi were sectioned just distal to their bifurcation. We also studied 10 control subjects (5 female and 5 male, aged 26–46 years) who were healthy and had no history of significant respiratory or neurological illness. The heights of the transplant and control subjects respectively ranged from 157 to 179 cm (mean ± S.E.M., 169 ± 3 cm) and 157 to 192 cm (mean, 172 ± 3 cm) and their weights ranged from 43 to 75 kg (mean, 66 ± 6 kg) and from 55 to 80 kg (mean, 68 ± 3 kg). Subjects were comfortably seated for all procedures. Informed written consent was obtained from the subjects and the procedures were approved by the local ethics committee and conformed to the Declaration of Helsinki.

Lobeline and saline injections

A cannula was inserted in the antecubital vein at the right elbow and extension tubing connected (1.1 ml deadspace) so that injections could be made remotely. Subjects were unaware of the timing of the injections. The lobeline HCl (Clinalfa, Switzerland) was prepared in saline at a concentration of 1 mg ml^{-1}. Injections of either saline or lobeline (3 different doses) were delivered in a pseudo-random order to each subject. The lobeline was always given in increasing doses but these injections were randomly interspersed with an injection of saline as a control. The deadspace of the tubing was primed with the appropriate solution in advance of injection without the subject's knowledge. The timing and duration of injections were marked using a switch activated by an experimenter. Latencies were measured from the onset of injections. Instructions to subjects were read from a prepared script and all extraneous visual and auditory stimuli were minimised to ensure that the level of attention was similar for each injection and for each subject (see Gandevia *et al.* 1998*b*).

The doses of lobeline ranged from 18 to 60 μg kg^{-1}. In a previous study, using identical procedures, we determined the threshold dose for respiratory sensations in healthy controls to be ~18 μg kg^{-1} and the threshold dose for evoking cough to be ~36 μg kg^{-1} (Gandevia *et al.* 1998*b*). Subjects usually received three different doses of lobeline of ~20, 40 and 60 μg kg^{-1} (termed low (< 25 μg kg^{-1}), intermediate (30–45 μg kg^{-1}) and high (52–60 μg kg^{-1}) doses (see Table 1). Intervals of 5–10 min separated injections and up to three to five injections of lobeline were performed in a session. All control subjects ($n = 10$) received low, intermediate and high doses, while for transplant subjects 5 of 8 received low doses, all 8 received intermediate doses and 6 of 8 received high doses (see Table 1 and Fig. 3).

A Doppler method was used to estimate the transit time for an injected solution to reach the right atrium from the injection site at the elbow (Michenfelder *et al.* 1972). The right atrium was targeted with a dual-frequency ultrasound probe positioned parasternally in the right third intercostal space (Parks Medical Electronics, model 915-AC, Aloha, OR, USA). A characteristic change in the auditory signal occurred shortly after injection of a mixture of 1–3 ml of saline with fine microbubbles. This occurred at 2.3–3.5 s following injection in both control and transplant subjects (mean 3 s).

Measurements of responses to lobeline injection

To determine the onset and intensity of any sensations associated with breathing, each subject signalled with a rotary potentiometer operated by the left hand. In some studies, the onset of other sensations associated with the injections (such as hot flushes or nausea) were signalled by an additional transient movement of the potentiometer. At least 2 min after each injection the subject was asked to describe any respiratory sensations and the responses were transcribed from tape recording. The subject then selected evoked sensations from a list (based on sensations reported by Raj *et al.* 1995; as used by Gandevia *et al.* 1998*b*) and indicated their apparent location (nose, throat, larynx, upper or lower chest, or elsewhere). The listed sensations (in order) included: (1) 'air hunger', (2) 'tightness in the chest or elsewhere', (3) 'difficulty or discomfort with breathing', (4) 'choking', (5) 'wheezing', (6) 'pain or burning in the chest or elsewhere', (7) 'swallowing', (8) 'smoke in the throat', (9) 'a need to cough', and (10) 'any other sensations'. To estimate the peak intensity of respiratory discomfort for each selected sensation, subjects selected a number from a modified 10-point category scale (Borg, 1982) as used in other studies of respiratory discomfort (e.g. Gandevia *et al.* 1993, see Fig. 3 legend).

We monitored blood pressure, heart rate and ventilation. Blood pressure was measured continuously using a plethysmograph-based system from a finger of the right hand (Ohmeda 2300, Finapres, WI, USA). Ventilation was measured non-invasively with a pair of calibrated inductance bands positioned around the upper chest and abdomen. The gains of the signals were adjusted using the isovolume manoeuvre (Chadha *et al.* 1982). In addition, oxygen saturation was measured with an oximeter which also provided heart rate (averaged over 5 beats). End-tidal levels of CO_2 were monitored via nasal prongs (Ametek, Pittsburgh, PA, USA). All signals were recorded on tape (Vetter PCM) and simultaneously sampled at 100 Hz and stored to disk (Cambridge Electronic Design 1401+, Spike 2, Cambridge, UK) for subsequent analysis.

Table 1. Subject data

Subject	Transplant	Time since operation (months)	Prior disease	Lobeline doses (μg kg^{-1})
1M	BLT	0.5	Bronchiectasis	20, 40, 60
2M	HLT	0.5 (and 31)	Ventricular septal defect	20, 40, 60
1F	BLT	3	Bullous emphysema	30, 60
2F	HLT	4	Post-partum hypertension	30, 60
3M	BLT	5	Emphysema	18, 30, 42
4M	BLT	12	Cystic fibrosis	20, 40, 60
3F	HLT	13	Bronchiectasis	30, 43, 52, 60
5M	HLT	90	Sarcoidosis	20, 20, 40, 40
6M	HT	2	Cardiomyopathy	20, 40, 40, 60
7M	HT	2.5	Cardiac failure	20, 40, 60, 80
Controls ($n = 10$)	—	—	—	20, 40, 60

Subject data for control subjects (bottom line) and individual subjects with bilateral lung transplants (BLT), heart and bilateral lung transplants (HLT) and heart transplants alone (HT). Male subjects are indicated by 'M' and female subjects by 'F'. Data include time since transplant operation, prior disease and lobeline doses delivered in the study.

Analysis of sensations

For the sensory responses, the latency of the onset of any sensations after the start of the injection and their time course were derived from the potentiometer signal and were measured with cursors. Peak intensity of each sensation was taken from the subjective response reported on the 10 point Borg scale. The sensory descriptors were divided into two categories: (i) those related to a noxious sensation in the chest or throat (sensations labelled 6, 8 and 9, see above under 'Measurement of responses to lobeline injection') and (ii) those related to breathlessness or 'air hunger' (sensations labelled 1, 2 and 3, see above). This allowed a comparison of the two distinct types of sensation. The Borg score for each sensation within each of the two subgroups was then summed to give total scores – the higher the score, the more severe the sensations related to the lobeline injection (maximum possible score = 30).

Sensations 4, 5 and 7 were not formally included in the analysis. Sensation 5 was not reported by any subject. Sensations 4 and 7 were only ever reported by control subjects. They were not included since a sensation of 'choking' could be related to breathlessness as well as respiratory discomfort, and the presence of 'swallowing' was not always accurately reported by the subjects.

Analysis of cardiorespiratory variables

Changes in heart rate were assessed from the signal of heart rate averaged over five beats. Blood pressure changes were assessed on a beat-to-beat basis. Mean arterial pressure (MAP) was calculated from systolic and diastolic pressures with a standard formula (1/3(systolic − diastolic) + diastolic). Minute ventilation was calculated on a breath-by-breath basis from the calibrated chest and abdominal inductance bands and hyperventilation was defined by a decrease in end-tidal CO_2.

Average heart rate, systolic, diastolic and mean arterial pressures, and minute ventilation were measured before the onset of the injection. The onset time of changes in these variables was determined using cursors. For heart rate and blood pressure, the amplitude of any changes was measured. The average level of ventilation between ∼20 and 60 s after the injection was expressed relative to the average level before the injection.

An early respiratory event was defined as the occurrence of hypopnoea, apnoea or a cough at the same time that the subject signalled the onset of sensations.

Statistical analyses

All key comparisons were made using non-parametric statistical tests. Spearman's rank correlation was used to relate the timing and intensity of the sensations to all delivered doses of lobeline. A 2-way analysis of variance (ANOVA) on ranks was used to assess the differences in Borg score for the sensory variables. If a significant difference was detected between control and transplant subjects a Mann-Whitney U test was used to determine at which doses the difference occurred. Fisher's exact tests were used to compare the number of subjects in each group that experienced lobeline-related sensations, or had changes in cardiovascular and respiratory variables. Statistical significance was set at the 5% level. Significant changes in MAP were defined by a change outside twice the interquartile range of the pre-injection values. Unless otherwise stated, data are given as median and interquartile ranges (IQ range). All statistical analyses (except regression) were performed on one value per subject for each dose. Responses were averaged if subjects received more than one injection at the same dose. The linear regression and mean (± S.E.M.) are used for illustrative purposes only in parts of Fig. 2.

RESULTS

Sensory responses

In contrast to subjects with bilateral lung transplants, all control subjects ($n = 10$) reported a noxious sensation in the throat, larynx and upper chest. Most control subjects (8 of 10) also reported a later sensation of breathlessness following injections of lobeline (Figs 1*A*, 2*A* and 3). Both the noxious sensation and the sensation of breathlessness increased in intensity with increasing dose of lobeline (Spearman's rank correlation; each $P < 0.05$). There were no false positive reports of respiratory sensations in any subject when saline had been injected or when no injection

had occurred. As noted previously in control subjects (Gandevia *et al.* 1998*b*), the higher the dose of lobeline, the longer the duration of respiratory discomfort and the shorter the onset latency of sensations (each $P < 0.05$) (Fig. 2*B* and *C*, left panels). However, in the subjects with bilateral lung transplants, there was no correlation between the dose of lobeline and the onset time of sensation ($P = 0.3$) or the duration of the evoked sensations ($P = 0.5$) (Fig. 2*B* and *C*, right panels).

The full range of sensations and their intensities are shown for all subjects and doses in Fig. 3. The sensations are ordered such that they are divided into subgroups (noxious sensations on the left and sensations of breathlessness on the right). In this figure, the larger the size of each symbol the higher the intensity of the sensation. It suggests that the occurrence and intensity of the sensations evoked by injection of lobeline were greater in control subjects compared with transplant subjects at all doses. The formal analysis is depicted in Fig. 4 for the initial noxious sensation, the sensation of breathlessness and the respiratory effects of the 'stimuli'. At the three doses, the intensity of the noxious sensations was significantly greater in control than transplant subjects (ANOVA on ranks, $P < 0.001$). The tendency for sensations of breathlessness to have a higher intensity rating in control subjects was not statistically significant for each dose. For the transplant subjects there was only a weak positive correlation between dose and sensations related to breathlessness or 'air hunger' ($P < 0.05$) but no correlation between dose and the noxious sensation in the chest and throat ($P = 0.2$). Both control and transplant subjects localised the sensations to the throat, upper and lower chest while only control subjects reported sensations in the nose, mouth and larynx.

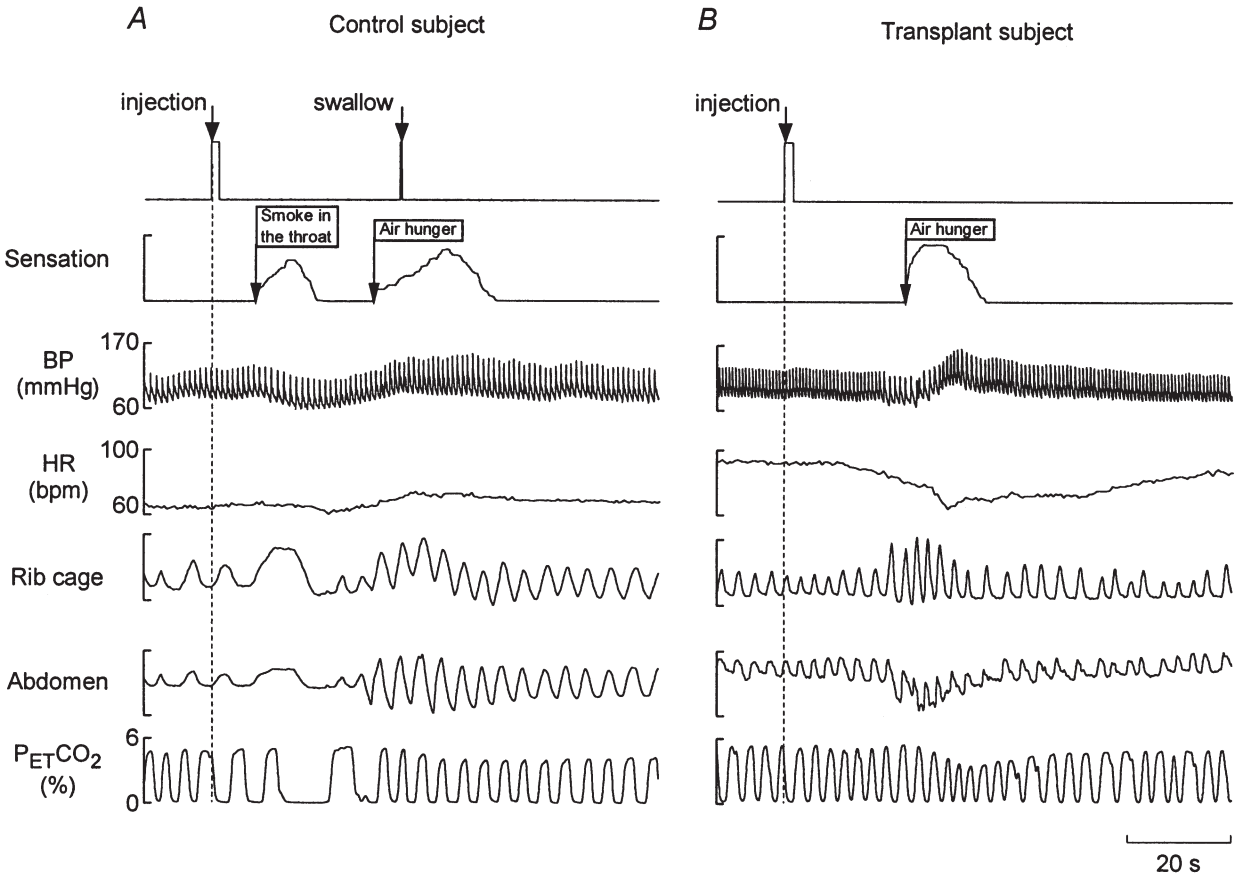

Figure 1. Responses to injection of lobeline in a control and a transplant subject

Typical examples of responses to injections of the intermediate dose of lobeline HCl (40 μg kg^{-1}) from a control subject (*A*) and a subject with a bilateral lung transplant (*B*, subject 1M in Table 1). Top trace shows the timing of the injection and any swallow or cough. Second trace is from the potentiometer used to signal sensations associated with the injection of lobeline. The earliest sensations were the noxious sensations (e.g. *A*, 'smoke in the throat'). The control subject also showed a brief apnoea. Both subjects signalled a sensation of 'air hunger' which was accompanied by a period of hyperventilation. The hyperventilation resulted in a reduction of the end-tidal CO_2 (bottom trace). Changes in heart rate (HR (bpm, beats min^{-1})) and blood pressure (BP) are also evident in each subject.

Cardiorespiratory responses

In addition to the sensory changes, lobeline changed ventilation, blood pressure and heart rate. For control subjects, an early respiratory 'event' (defined as a cough or apnoea, see Methods) occurred in all subjects for the intermediate (6 apnoeas, 4 coughs) and high doses (3 apnoeas, 7 coughs) of lobeline. At these doses only one of 8 transplant subjects studied at the intermediate dose (cough) and 3 of 6 transplant subjects studied at the high dose had early respiratory events (2 apnoeas, 1 cough), a significant difference between the groups (Fisher's exact test, $P < 0.05$ for both doses; Fig. 4*C*). The brief apnoea or

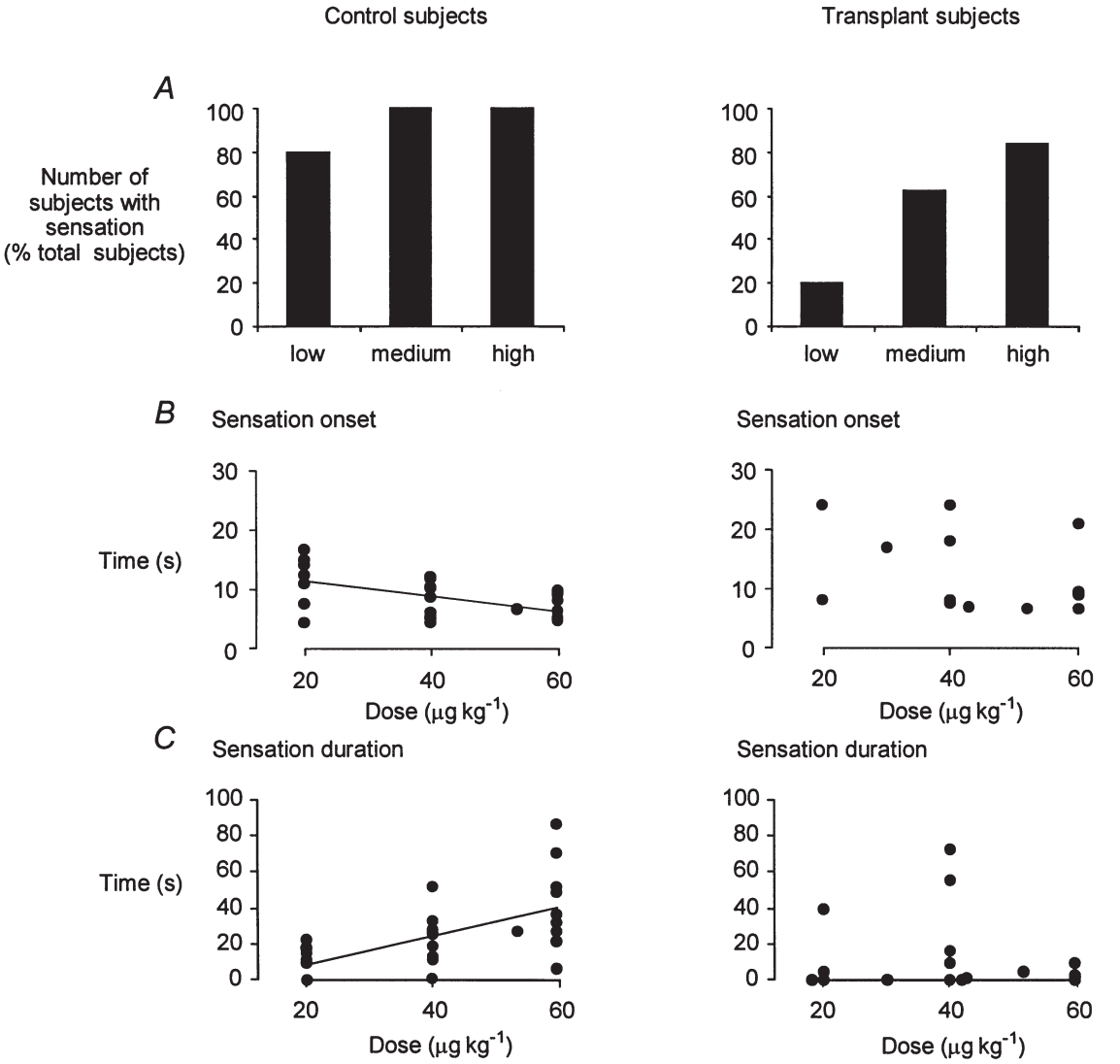

Figure 2. Effect of different strength doses on the onset time and duration of evoked sensations

A, incidence of evoked sensations to different doses of lobeline (low, intermediate and high) in control subjects (left panel) and lung transplant subjects (right panel). Both noxious sensations and sensations of breathlessness are included. Each subject is represented once for each dose. *B*, onset latency of first evoked sensations plotted against the dose of lobeline (μg kg^{-1}) for all subjects at all injected doses (some subjects are represented more than once at the same or similar doses). For control subjects (left panel) there is a significant negative correlation between onset of sensations and dose of lobeline ($P < 0.05$), while for the transplant subjects (right panel) there is not ($P = 0.3$). *C*, duration of evoked sensations plotted against the dose of lobeline for all subjects. For control subjects (left panel) there is a significant positive correlation between duration of sensation and dose of lobeline ($P < 0.05$), while for the transplant subjects (right panel) there is not ($P = 0.5$). Occasionally some doses of lobeline produced sensations lasting less than 1 s and the relevant points appear close to zero in the panel. Lines represent the significant linear correlations. Note that some subjects did not signal sensations at some doses. These data are represented in the graphs in *C* as points at time zero but are not included in the statistical analyses of correlations.

hypopnoea usually lasted about 2–4 s and in control subjects was usually associated with a swallowing movement. The timing of the early respiratory events commonly coincided with the onset of induced sensations.

In the majority of control and transplant subjects, a long-latency hyperventilation occurred in response to injections of lobeline (Fig. 4*D*). This is likely to reflect the stimulation of remote arterial chemoreceptors by systemic circulation of the lobeline. The hyperventilation was accompanied by a sensation of breathlessness in most subjects (Fig. 4*B*). On average, ventilation increased 2- to 3-fold compared with the pre-injection level in both control and transplant subjects with the intermediate and high doses of lobeline. There was no difference in magnitude between subject groups ($P = 0.5$). At high doses the ventilatory increases occurred at 25 s (IQ range 20–27 s) for control subjects and at 16 s (14–20 s) for transplant subjects ($P = 0.14$).

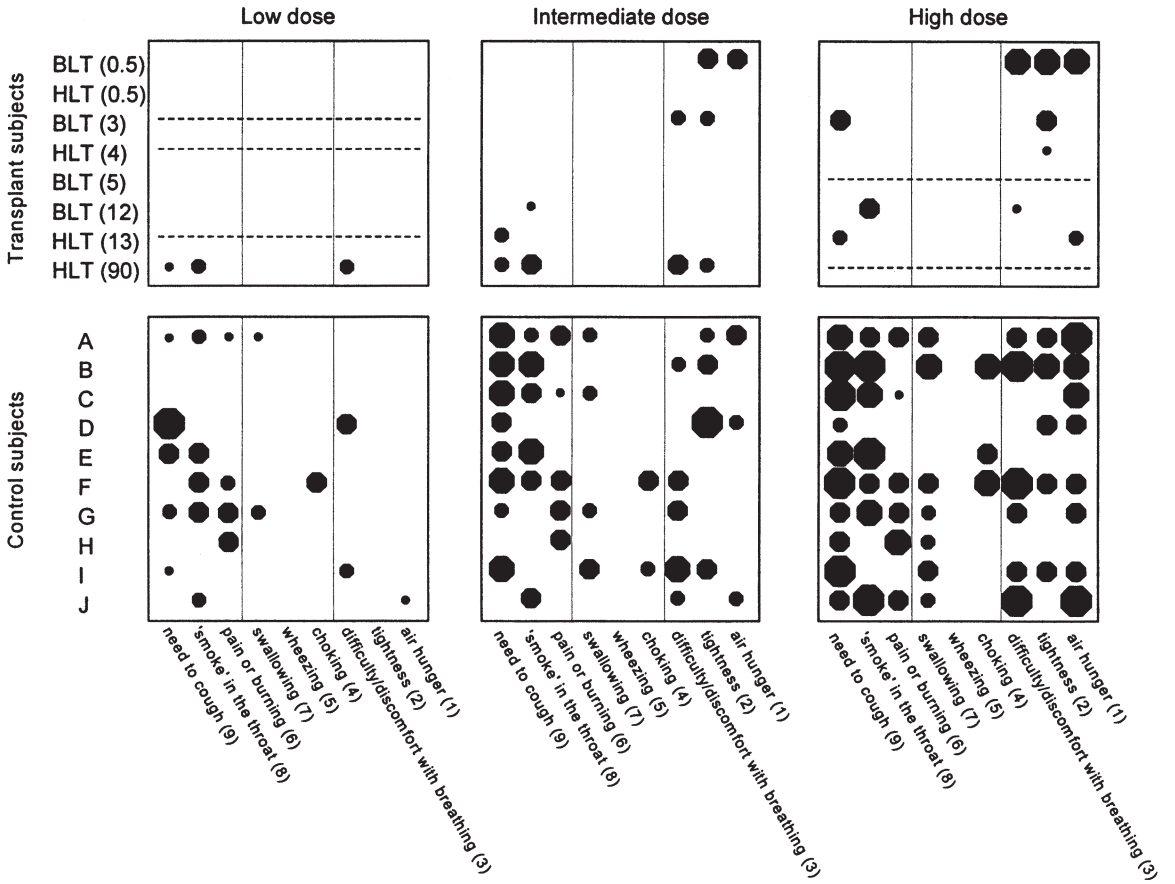

Figure 3. Individual reports of sensation type and intensity for each dose

Representation of reports from individual subjects of sensation in response to low, intermediate and high doses of lobeline. For transplant subjects (three top panels), time since lung transplantation (months) and type of transplant (BLT or HLT) are indicated (at left). Transplant subjects are ranked from shortest to longest time since transplantation from the top to the bottom row. Data from control subjects are shown in the three bottom panels. Sensation descriptors are labelled along the bottom and are divided into noxious sensations (left column), sensations of breathlessness (right column) and other sensations (middle column). The size of each octagon represents the intensity of the sensation as reported by each subject on the 10 point Borg scale. There are 5 sizes of octagon representing Borg score ranges. The octagons from smallest to largest represent scores of 0.5 and 1; 1.5, 2 and 2.5; 3, 3.5 and 4; 4.5, 5 and 5.5; and 6, 6.5, 7 and 8 respectively (see below), and thus the larger the octagon the more intense the sensation. Blank spaces represent no sensation. Dashed lines represent subjects not studied at that dose. One representative injection per subject for each dose is shown.

Borg score descriptors:				
	10	Extremely large (max.)	4	Considerable
	9		3	Moderate
	8		2	Mild
	7	Very large	1	Slight
	6		0.5	Just noticeable
	5	Large	0	Infinitely small

Ventilation returned to pre-injection level about 1–2 min after the hyperventilation began (see Fig. 1*A*).

In response to injections of lobeline, we observed a significant decrease in blood pressure beginning at about 12–15 s in all control subjects. The mean arterial pressure decreased after injection of lobeline for the intermediate dose (by 23 mmHg; 17–27 mmHg (median; IQ range)) and for the high dose (by 30 mmHg; 22–42 mmHg). By contrast, two of the six transplant subjects showed a significant decrease in mean arterial pressure after injection of lobeline at the high dose and this incidence is significantly less than in control subjects ($P < 0.05$; Fig. 5*A*). One transplant subject showed a small increase in MAP in response to lobeline injections.

The changes in heart rate after lobeline injection were complex and no clear patterns emerged (Fig. 5*B*). At the intermediate dose, heart rate increased by 10–33 beats min^{-1} (median increase 22 beats min^{-1}) in eight control subjects with a latency of 15–30 s and by 5–13 beats min^{-1} (median increase 9 beats min^{-1}) in two transplant subjects. Heart rate decreased in two control subjects (by 7 and 9 beats min^{-1}) and in two transplant subjects (by 7 and 30 beats min^{-1}). There was no significant difference in the cardiovascular responses to lobeline injection between subjects with bilateral lung transplants and those with both heart and bilateral lung transplants. However, the small numbers may mask subtle differences.

Responses to lobeline in subjects with heart transplant alone

The responses to lobeline injections were also tested in two subjects who had undergone heart transplants alone. These subjects were on a similar regimen of immunosuppression and antimicrobial medications and were viewed as a 'patient' control group for this reason. The

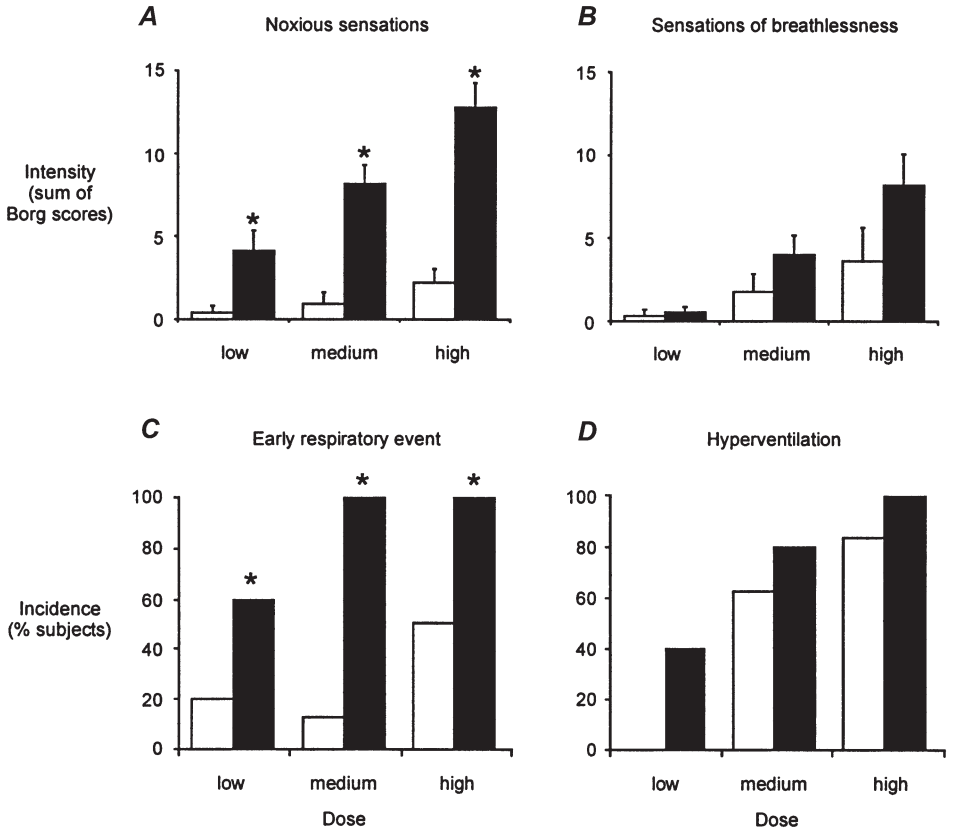

Figure 4. Intensity of evoked sensations and incidence of respiratory responses associated with different doses of lobeline

Intensity of evoked sensations (*A* and *B*, mean ± S.E.M.) and incidence of corresponding respiratory responses (*C* and *D*) associated with low, intermediate and high doses of lobeline. □, data from transplant subjects; ■, data from control subjects. There was a significantly higher intensity of noxious sensation and a higher incidence of early respiratory events experienced by the control subjects at all doses (*A* and *C*). There was no significant difference between control and transplant subjects in the intensity of the breathless sensation or in the incidence of hyperventilation (*B* and *D*). Note that the intensity of the two types of respiratory sensation is expressed as the sum of three Borg ratings and the maximal possible score is 30 (see Methods). * Significant difference between groups ($P < 0.05$). One value per subject for each dose was used in the analysis.

sensations produced by lobeline injection in these subjects were similar to those in control subjects. The subjects reported sensations of breathlessness (Borg score for each subject, 5 and 2.5) and noxious sensations (Borg score, 6.5 and 2.5) localised to the throat, upper and lower chest. Both heart transplant subjects showed a decrease in blood pressure in response to lobeline. One of these subjects coughed, hyperventilated and increased heart rate after injections.

Results in subjects with long-term bilateral lung transplants

Up to this point, data from all lung transplantation subjects have been considered together. However, at intermediate doses, noxious sensations after the injection of lobeline were experienced by three of the lung transplant subjects. These three subjects were all studied 12 months or more after transplantation. At the high dose, a noxious sensation was also experienced by one other bilateral lung transplant subject studied only 3 months post-transplantation. One possible explanation is that afferent reinnervation of the lungs may occur. To investigate this possibility, one subject who was studied 2 weeks post heart and bilateral lung transplantation and who had not reported any sensations in response to lobeline injections was tested 31 months post-transplant using similar procedures. In contrast to the initial study, in the second study the subject reported sensations associated with the intermediate and high doses of lobeline. At the high dose (60 μg kg^{-1}) he signalled a noxious sensation (at 12 s) and breathlessness (at 26 s) and experienced marked hyperventilation (Fig. 6). The noxious sensation consisted of 'choking in the throat' and 'a need to cough' (Borg score = 3 for each). The subject also experienced breathlessness including 'tightness localised to both the chest and the throat', 'difficulty with breathing' and 'air hunger' (Borg score = 6 for each). The total duration of lobeline-induced sensations was 71 s.

Other sensations

Subjects also reported other sensations associated with the injection of lobeline. These occurred after the initial noxious sensations. They included nausea and hot flushes of the face, neck and shoulders. Five of ten control subjects and one transplant subject reported nausea ($P = 0.3$). Five of ten control subjects and six of eight transplant subjects reported a hot flush after the injection of lobeline ($P = 0.4$).

DISCUSSION

This study shows definitively that receptors in the human lungs are activated by suprathreshold doses of lobeline and this leads to noxious respiratory sensations. In recent lung transplant subjects no noxious respiratory sensations were reported after lobeline injections. In transplant subjects studied 12 months or more post-transplantation some noxious sensations were reported although of less intensity than in the control subjects. This study also suggests that after 1–2 years there may be functional reinnervation of the lungs after complete denervation associated with bilateral lung transplantation.

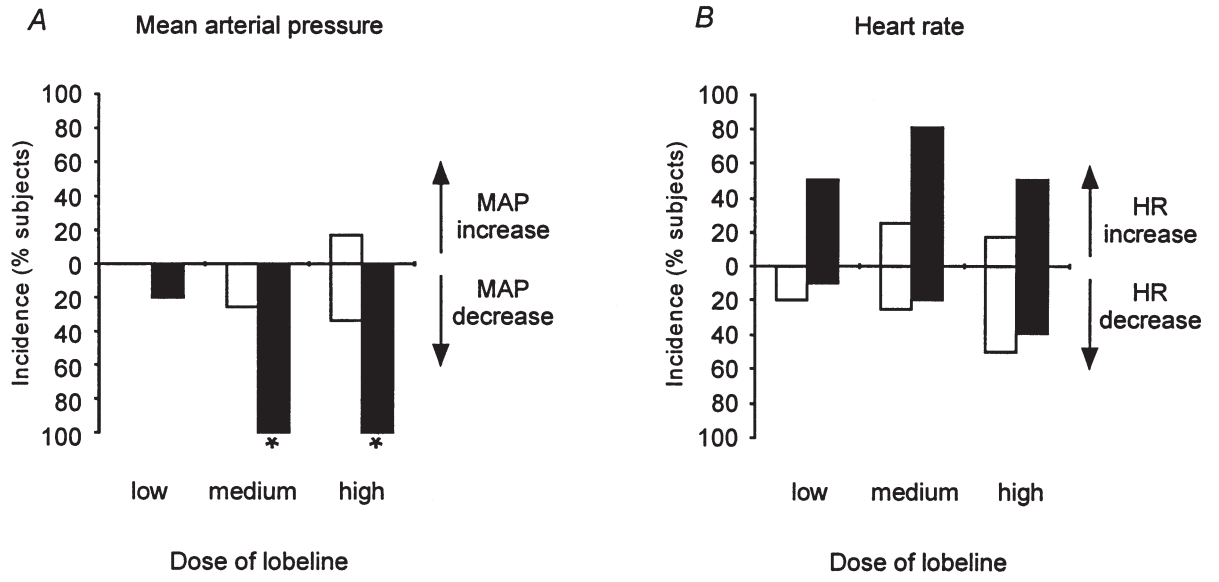

Figure 5. Incidence of cardiovascular changes associated with different doses of lobeline

Incidence of changes in mean arterial pressure (MAP; *A*) and heart rate (HR, *B*) with low, intermediate and high doses of lobeline. Graphs depict increases (upwards columns) and decreases (downwards columns) in MAP and heart rate. ■, data from control subjects; □, data from transplant subjects. There was a significantly higher incidence of decreased MAP in the control subjects at the intermediate and high doses of lobeline. * Significant difference between groups ($P < 0.05$). The incidence of heart rate changes and their direction did not differ between control and transplant subjects. One value per subject for each dose was used in the analysis.

There have been many investigations into the mechanism of the sensations and cough in response to lobeline injection in humans. Lobeline was commonly used in patients to assess circulation time on the basis of the time it took for subjects to experience cough and hyperventilation after injection (Robb & Weiss, 1934; Berliner, 1940; Bevan & Murray, 1963; Stern *et al.* 1966). The receptors which caused the cough in humans were first believed to be in the systemic circulation but were later localised to an area supplied by the pulmonary circulation (Eckenhoff & Comroe, 1951; Bruderman *et al.* 1966; Stern *et al.* 1966; see also Dawes *et al.* 1951). Injections of lobeline into the left chambers of the heart in humans do not cause cough but can cause hyperventilation, whereas injections into the descending aorta cause no changes in ventilation (Stern *et al.* 1966). However, the sensations experienced by patients after injections of lobeline were initially postulated to be caused by stimulation of receptors in the pleura or in the bronchi (Eckenhoff & Comroe, 1951). More recently, Raj *et al.* (1995) tested the threshold for sensations resulting from injections of lobeline in human subjects. They suggested that the afferents responsible for the noxious sensations were pulmonary C fibres as the threshold for sensation did not change after sensitisation of rapidly adapting pulmonary receptors. Our study strongly supports a role for pulmonary afferents which are accessible to the pulmonary circulation in the production of short-latency noxious respiratory sensations such as burning or smoke in the chest and throat, and a need to cough. The data suggest that the full duration of the sustained noxious sensation requires intact pulmonary vagi. Although most lung transplant subjects reported sensations of 'breathlessness' at the same intensity as control subjects, only half ever reported noxious sensations although at a lower intensity and at a higher threshold than control subjects. We suggest that some pulmonary reinnervation may account for the low intensity noxious sensations experienced by these subjects. Consistent with this, when one transplant subject who had initially felt no sensations was studied 2.5 years later, typical sensations associated with pulmonary C fibre stimulation by lobeline had returned. While there has previously been little evidence for reinnervation in human lung transplant recipients

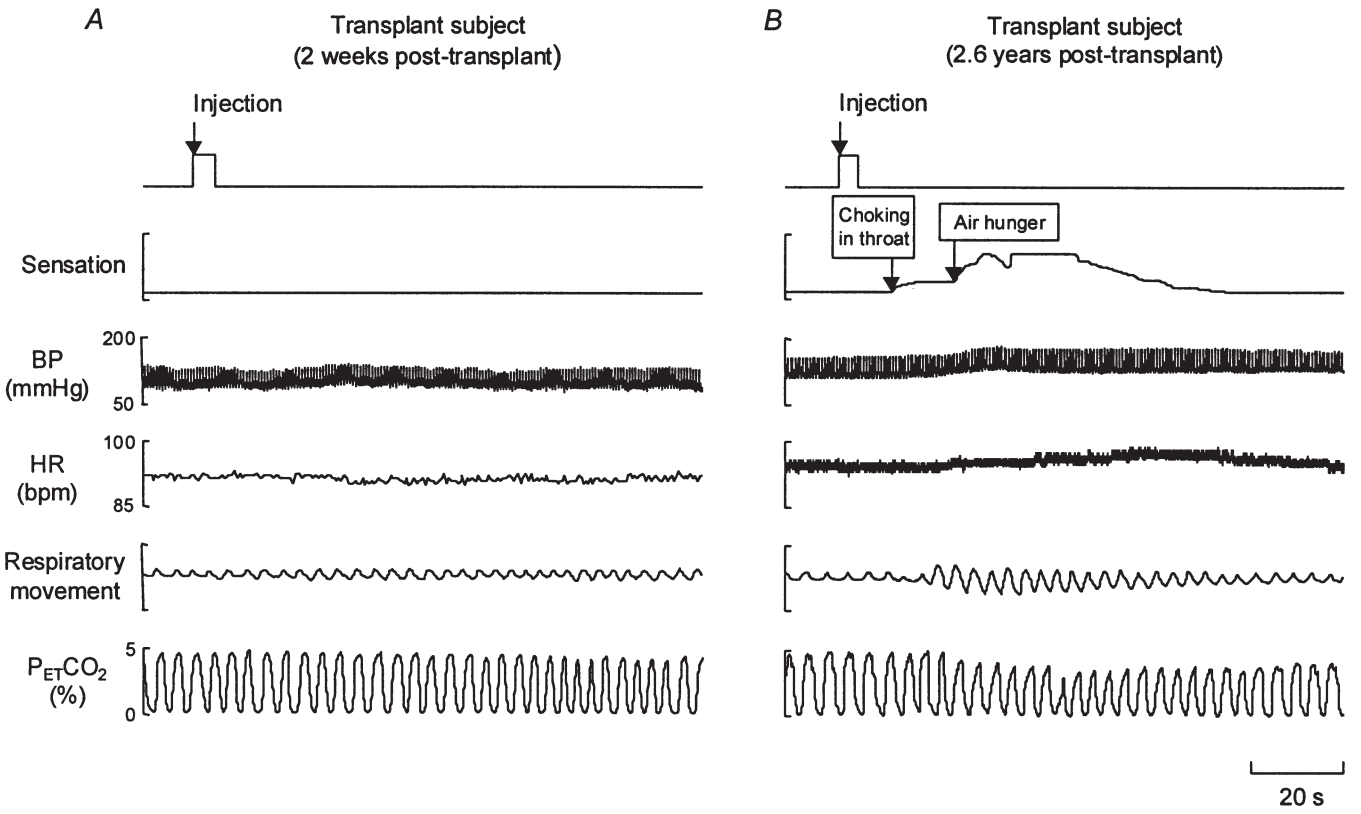

Figure 6. Two studies in one transplant subject

Data from a heart and bilateral lung transplant subject studied at 2 weeks (*A*) and 2.6 years after the transplantation (*B*) (subject 2M in Table 1). Top traces show the timing of the injections. Second trace is from the potentiometer used to signal sensations associated with the injection of lobeline. The subject signalled no sensations on the first occasion but signalled both noxious sensations and sensations of breathlessness in response to the same dose (60 μg kg^{-1}) of lobeline 2.6 years after the surgery. Sensations of air hunger were associated with a period of hyperventilation as indicated by the increased respiratory movement (fifth trace) and the subsequent decrease in end-tidal CO_2 (sixth trace).

prior to 12 months post-transplantation (Higenbottam *et al.* 1989; Hathaway *et al.* 1993; Iber *et al.* 1995; cf. Ramaekers *et al.* 1996), pulmonary reinnervation has been reported after about 8 months in allograft transplanted rats (Kawaguchi *et al.* 1998), 5 months in autotransplanted dogs (Mattila *et al.* 1987) and 2 months in autotransplanted monkeys (Mihm *et al.* 1989). Membrane receptors sensitive to lobeline have been reported in experimental neuromas (Leah *et al.* 1988), but in those transplant subjects who felt noxious sensations, the latency after lobeline injection suggests that the receptors were accessible to the pulmonary circulation. Alternatively, it is possible that recovery of sensitivity to lobeline may have occurred through changes in intact pathways over time. Sensitivity of non-pulmonary receptors may have increased or plastic changes may have occurred in the CNS (e.g. Pan *et al.* 1998). Changes secondary to altered pulmonary perfusion following angiogenesis may also be relevant.

The intensity of the breathless sensation and the incidence of hyperventilation in response to lobeline injection were not significantly different between control and transplant subjects. This suggests that receptors which cause the hyperventilation are located neither in the lungs nor the heart, but elsewhere in the systemic circulation such as in the carotid body. Overall, based on the apparent dissociation of the noxious sensations and the sensations of breathlessness in the transplant subjects, our results suggest that the noxious sensations are due to the stimulation of pulmonary afferents (most likely to be pulmonary C fibres) whereas the sensations of breathlessness probably depend on the activation of arterial chemoreceptors.

Although heart rate changes were variable across subjects, there was a consistent decrease in mean arterial pressure in control subjects in response to the injection of lobeline that was not consistent across transplant subjects. From these data, we suggest that the lobeline-induced decrease in mean arterial pressure is a reflex response to the stimulation of pulmonary afferents.

The cardiovascular changes associated with injections of lobeline in human subjects have been observed during the early period of ventilatory depression or apnoea (Bevan & Murray, 1963). However, blood pressure and heart rate changes have not always been observed in humans probably because of the dosage delivered (Jain *et al.* 1972; Raj *et al.* 1995). In contrast to lobeline, phenyldiguanide causes no noxious sensations in human subjects but does cause reflex hypotension, bradycardia and hyperventilation, presumably by stimulation of carotid body chemoreceptors rather than pulmonary receptors (Jain *et al.* 1972).

We conclude that in human subjects, the noxious respiratory sensation, decrease in mean arterial pressure and early respiratory events such as apnoea and cough associated with an intravenous injection of lobeline require intact pulmonary afferents. Therefore, we suggest that the entire period of the noxious sensations most likely corresponds to a period of stimulation of pulmonary but not bronchial lobeline-sensitive receptors. Finally, we have presented some evidence for reinnervation of pulmonary afferents in subjects who have undergone bilateral lung transplantation.

Anand, A. & Paintal, A. S. (1980). Reflex effects following selective stimulation of J receptors in the cat. *Journal of Physiology* **299**, 553–572.

Berliner, K. (1940). Use of alpha lobeline for measurement of velocity of blood flow. *Archives of Internal Medicine* **65**, 896–901.

Bevan, J. A. & Murray, J. F. (1963). Evidence for a ventilation modifying reflex from the pulmonary circulation in man. *Proceedings of the Society for Experimental Biology and Medicine* **114**, 393–396.

Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise* **14**, 377–381.

Bruderman, I., Stern, S. & Braun, K. (1966). Respiratory effects of intravenous lobeline in normal subjects and in patients with mitral stenosis. *British Heart Journal* **28**, 740–745.

Chadha, T. S., Watson, H., Birch, S., Jenouri, G. A., Schneider, A. W., Cohn, M. A. & Sackner, M. A. (1982). Validation of respiratory inductive plethysmography using different calibration procedures. *American Review of Respiratory Disease* **125**, 644–649.

Dawes, G. S., Mott, J. C. & Widdicombe, J. G. (1951). Respiratory and cardiovascular reflexes from the heart and lungs. *Journal of Physiology* **115**, 258–291.

Deshpande, S. S. & Devanandan, M. S. (1970). Reflex inhibition of monosynaptic reflexes by stimulation of type J pulmonary endings. *Journal of Physiology* **206**, 345–357.

Eckenhoff, J. E. & Comroe, J. H. Jr (1951). Blocking action of tetraethylammonium on lobelin-induced thoracic pain. *Proceedings of the Society for Experimental Biology and Medicine* **76**, 725–726.

Gandevia, S. C., Butler, J. E., Anand, A., Crawford, M. R., Glanville, A. R., Paintal, A. S., Taylor, J. L. & McKenzie, D. K. (1998*a*). Sensations attributable to intrapulmonary afferents: Responses to lobeline in control subjects and patients with lung transplants. *Proceedings of the Australian Neuroscience Society* **9**, 74P.

Gandevia, S. C., Butler, J. E., Taylor, J. L. & Crawford, M. R. (1998*b*). Absence of viscerosomatic inhibition with injections of lobeline designed to activate human pulmonary C fibres. *Journal of Physiology* **511**, 289–300.

Gandevia, S. C., Killian, K., McKenzie, D. K., Crawford, M., Allen, G. M., Gorman, R. B. & Hales, J. P. (1993). Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. *Journal of Physiology* **470**, 85–107.

Ginzel, K. H. (1973). Muscle relaxation by drugs which stimulate sensory nerve endings – I. The effect of veratrum alkaloids, phenyldiguanide and 5-hydroxytryptamine. *Neuropharmacology* **12**, 133–148.

Ginzel, K. H. & Eldred, E. (1969). Relief of decerebrate rigidity by viscero-somatic reflex action. *Proceedings of the Western Pharmacological Society* **12**, 41–44.

Ginzel, K. H. & Eldred, E. (1970). A possible physiological role for the depression of somatic motor function by reflexes from the cardiopulmonary region. *Proceedings of the Western Pharmacological Society* **13**, 188–191.

Hathaway, T. J., Higenbottam, T. W., Morrison, J. F. J., Clelland, C. A. & Wallwork, J. (1993). Effects of inhaled capsaicin in heart-lung transplant patients and asthmatic subjects. *American Review of Respiratory Disease* **148**, 1233–1237.

Higenbottam, T., Jackson, M., Woolman, P., Lowry, R. & Wallwork, J. (1989). The cough response to ultrasonically nebulised distilled water in heart-lung transplantation patients. *American Review of Respiratory Disease* **140**, 58–61.

Iber, C., Simon, P., Skatrud, J. B., Mahowald, M. W. & Dempsey, J. A. (1995). The Breuer-Hering reflex in humans. Effects of pulmonary denervation and hypercapnia. *American Journal of Respiratory and Critical Care Medicine* **152**, 217–224.

Jain, S. K., Subramanian, S., Julka, D. B. & Guz, A. (1972). Search for evidence of lung chemoreflexes in man: study of respiratory and circulatory effects of phenyldiguanide and lobeline. *Clinical Science* **42**, 163–177.

Kalia, M. (1973). Effects of certain cerebral lesions on the J reflex. *Pflügers Archiv* **343**, 297–308.

Kalia, M., Koepchen, H. P. & Paintal, A. S. (1973). Somatomotor and autonomous effects of type-J receptor stimulation in awake freely-moving and restrained cats. *Pflügers Archiv* **339**, R80.

Kawaguchi, A. T., Shirai, M., Yamano, M., Ishibashi-Ueda, H., Yamatodani, A. & Kawashima, Y. (1998). Afferent reinnervation after lung transplantation in the rat. *Journal of Heart and Lung Transplantation* **17**, 341–348.

Leah, J., Koschorke, G. M., Welk, E. & Zimmermann, M. (1988). The expression of sensory receptors on regenerating and regenerated cutaneous C fibres. *Progress in Brain Research* **74**, 341–348.

Mattila, S., Mattila, I., Viljanen, B. & Viljanen, A. (1987). Reappearance of Hering-Breuer reflex after bilateral autotransplantation of the lungs. *Scandinavian Journal of Thoracic Cardiovascular Surgery* **21**, 15–20.

Michenfelder, J. D., Miller, R. H. & Gronert, G. A. (1972). Evaluation of an ultrasonic device (Doppler) for the diagnosis of venous air embolism. *Anesthesiology* **36**, 164–167.

Mihm, F. G., Popovic, B. K., Noe, C., Hilberman, M. & Reitz, B. A. (1989). Recovery of cardiopulmonary reflexes in monkeys undergoing heart-lung transplantation. *Journal of Thoracic and Cardiovascular Surgery* **98**, 510–516.

Paintal, A. S. (1969). Mechanism of stimulation of type J pulmonary receptors. *Journal of Physiology* **203**, 511–532.

Paintal, A. S. (1970). The mechanism of excitation of type J receptors, and the J reflex. In *Breathing: Hering-Breuer Centenary Symposium*, ed. Porter, R., pp. 59–71. Churchill, London.

Paintal, A. S. (1973). Vagal sensory receptors and their reflex effects. *Physiological Reviews* **53**, 159–227.

Paintal, A. S. (1986). The visceral sensations – some basic mechanisms. *Progress in Brain Research* **67**, 3–19.

Paintal, A. S. (1995). Sensations from J receptors. *News in Physiological Sciences* **10**, 238–243.

Pan, L. G., Forster, H. V., Martino, P., Strecker, P. J., Beales, J., Serra, A., Lowry, T. F., Forster, M. M. & Forster, A. L. (1998). Important role of carotid afferents in control of breathing. *Journal of Applied Physiology* **85**, 1299–1306.

Pickar, J. G., Hill, J. M. & Kaufman, M. P. (1993). Stimulation of vagal afferents inhibits locomotion in mesencephalic cats. *Journal of Applied Physiology* **74**, 103–110.

Raj, H., Singh, V. K., Anand, A. & Paintal, A. S. (1995). Sensory origin of lobeline-induced sensations: a correlative study in man and cat. *Journal of Physiology* **482**, 235–246.

Ramaekers, D., Ector, H., Vanheaecke, J., van Cleemput, J. & van de Werf, F. (1996). Heart rate variability after cardiac transplantation in humans. *Pacing and Clinical Electrophysiology* **19**, 2112–2119.

Robb, G. P. & Weiss, S. (1934). The velocity of pulmonary and peripheral venous blood flow and related aspects of the circulation in cardiovascular disease. *American Heart Journal* **9**, 742–763.

Schiemann, B. & Schomburg, E. D. (1972). The inhibitory action of type J pulmonary receptor afferents on the central motor and fusimotor activity and responsiveness in cats. *Experimental Brain Research* **15**, 234–244.

Seals, D. R., Suwarno, N. O., Joyner, M. J., Iber, C., Copeland, J. G. & Dempsey, J. A. (1993). Respiratory modulation of muscle sympathetic nerve activity in intact and lung denervated humans. *Circulation Research* **72**, 440–454.

Stern, S., Bruderman, I. & Braun, K. (1966). Localization of lobeline-sensitive receptors in the pulmonary circulation in man. *American Heart Journal* **71**, 651–655.

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Corresponding author

S. C. Gandevia: Prince of Wales Medical Research Institute, Barker Street, Randwick, NSW 2031, Australia.

Email: s.gandevia@unsw.edu.au