Sensory origin of lobeline-induced sensations: a correlative study in man and cat

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1. Intravenous injections of lobeline HCl into twenty-six normal young male human volunteers produced sensations of choking, pressure or fumes in the throat and upper chest at a mean threshold dose of 12 μg kg^{-1}.

2. Reflex changes in breathing pattern usually appeared just before the sensations. Increasing the dose of lobeline increased the intensity of the sensations gradually until a dry cough appeared at a mean threshold dose of 24·3 μg kg^{-1}. At these doses there was a mean difference of 0·3 s in the latencies for sensation and respiratory reflex; in four subjects there was no difference at all.

3. In cats anaesthetized with 35 mg kg^{-1} sodium pentobarbitone, injecting 25–67 μg kg^{-1} lobeline into the right atrium sensitized thirteen out of seventeen rapidly adapting receptors (RARs). In three out of four cats lobeline had no excitatory effect on the RARs in the absence of normal activity (i.e. when it was injected while artificial respiration was suspended), but on restarting the respiration the activity increased greatly. After injecting lobeline, the activity increased during inflation or deflation or in both phases of the respiratory cycle. It also increased greatly during deflation produced by suction of air from the lungs after lobeline. Such presumed increased activity in the RARs of man produced by forced expiration to residual volume at the time lobeline-induced sensations were expected did not enhance the sensations in any subject.

4. In all the subjects tested, forced expiration alone, which should stimulate RARs, never produced a dry cough or sensations similar to those produced by lobeline.

5. The results suggest that since the reflex respiratory effects of lobeline are due to J receptors, the sensations and cough can also be attributed to them, since both events occur at about the same time, and also because the RARs, and the slowly adapting receptors (SARs), do not seem to play a primary role in producing or potentiating the sensations.

Following the observations of Jain, Subramanian, Julka & Guz (1972) it is now generally agreed (see Paintal, 1973; Coleridge & Coleridge, 1984; Karlsson, Sant'Ambrogio & Widdicombe, 1988) that the respiratory reflex effects which appear about 2 s after injecting lobeline into the pulmonary artery of man can be attributed to the stimulation of J receptors (also called pulmonary C fibres; Coleridge & Coleridge, 1984). However, apart from these reflex effects, lobeline injected in small doses (< 1 mg) also produces sensations of irritation localized mainly in the throat and upper chest. At higher doses the sensations are followed immediately by a dry cough. These sensations and the dry cough that follows have also been attributed to the stimulation of the J receptors by lobeline (Paintal, 1986*a*).

It is not known whether animals experience similar or different sensations when their J receptors are stimulated. However, conscious cats do not cough following intravenous injections of 150 μg phenyl diguanide (PDG) (Kalia, Koepchen & Paintal, 1973). Injections of such large doses of PDG must have led to large inputs from the J receptors (see Anand & Paintal, 1980). In view of this finding, even though the early respiratory reflex effects following injections of lobeline are due to inputs from the J receptors, it is possible that the sensations in the throat and the subsequent dry cough are not produced by J receptor inputs but by some other receptors. Clearly such receptors would have to be accessible to lobeline only through the pulmonary circulation and not the systemic circulation, since as shown by Stern, Bruderman & Braun (1966) no cough is produced by injecting lobeline into the left ventricle or a distal branch of the pulmonary artery (see Discussion). Are the receptors responsible for producing these sensations and the dry cough the rapidly adapting

receptors (RARs), since it is believed that the RARs produce coughing when they are stimulated (see Karlsson *et al.* 1988). We have attempted to answer this question through systematic observations on human volunteers and correlation of these observations with the responses of the RARs of cats, assuming that the RARs of man respond to lobeline in qualitatively the same way as those of cats. Therefore experiments were first carried out on cats to find out the responses of the RARs to lobeline. It is important, however, to keep in mind that the effects of drugs seen in cats may not be seen in man because there are marked species differences in the effects of drugs. For example, both PDG and 5-HT, which stimulate the J receptors of cats and thus produce the pulmonary chemoreflex, do not have any effect in dogs (see Coleridge & Coleridge, 1984).

The effects of different respiratory manoeuvres (designed to modify the discharges in slowly and rapidly adapting pulmonary receptors) on the sensations produced by lobeline were studied. In addition, since the sensations were felt mainly in the throat, the effect of irritating sensations in the throat produced by citric acid aerosol on the sensations produced by lobeline were also studied.

METHODS

Experiments on cats

Cats were anaesthetized with sodium pentobarbitone (35 mg kg^{-1}) given intraperitoneally; maintainance doses of 5–10 mg were given I.V. whenever needed. The chest was opened and they were artificially ventilated with a respiratory pump (Palmer 'Ideal') through a tracheal cannula (tip 2 cm distal to the larynx). The speed of the pump, which was routinely kept at about 17 cycles min^{-1}, was increased in some experiments (e.g. to 57 cycles min^{-1} in Fig. 1) in order to determine more precisely the latency for excitation of RARs by lobeline, since their activity was linked to inflation. The intratracheal pressure was recorded with a Statham type PM5 transducer. A positive end-expiratory pressure of 5 cmH_2O was maintained. This was withdrawn during certain observations (see Figs 1–3). The results reported were obtained from seventeen cats.

A catheter was inserted through the saphenous vein such that its tip lay in the right atrium. A second catheter was inserted into the left atrium through the left auricle; this was used for injecting phenyl diguanide (PDG) or lobeline into the left atrium. A third catheter was inserted through the femoral artery such that its tip lay in the thoracic aorta. This was used for recording the aortic pressure with a Statham type 23 dG transducer. A thermistor was inserted in some experiments through a branch of the pulmonary artery into the lower lobe of the left lung, such that its tip lay in the main pulmonary artery. This was used for recording the concentration of injected drugs in six cats using the method described earlier (Paintal & Anand, 1992). Briefly the method is based on the principle of relative dilution of multiple solutes in flowing fluids (Paintal & Anand, 1991). Using 'calories' as one of the solutes, the fall in temperature of the blood containing the drug was recorded and converted into the concentration of the drug in the blood by using a suitable equation. It was also used in the case of seventeen receptors for determining the latency of the responses of the receptors to injected drugs from the moment the drug arrived in the pulmonary artery to the beginning of stimulation. The position of the tip of the thermistor was examined postmortem and if it was jammed in the vessel wall the results were discarded. In those cats in which a thermistor could not be inserted or its tip was improperly located (see Paintal & Anand, 1992) the latencies in the case of eighteen receptors were measured from the signal provided by a foot switch. It was found that the foot switch signal preceded the arrival of the drug in the pulmonary artery by an average of 0·34 ± 0·1 s (mean ± S.D., $n = 25$). The signal was used for comparing latencies following right and left atrial injections.

Impulses from sensory receptors of the lungs were recorded using conventional techniques and set-up, i.e. the vagus nerve was separated out near the nodose ganglion and impulses were recorded from filaments of the nerve using an Isleworth type 102 preamplifier. The vagus nerve was stimulated low in the neck with a Devices isolated MK IV stimulator for determining the conduction velocities of the afferent fibres. The RARs were identified by their characteristic features, described by Knowlton & Larrabee (1946), consisting of sparse activity linked to the respiratory cycle, a high inflation threshold and an adaptation index of 80–100% (see also Widdicombe, 1954). No attempts were made to locate the receptors in the central or peripheral airways by mechanical probing. The slowly adapting receptors (SARs) were identified by their characteristic slowly adapting discharge to maintained inflation (Knowlton & Larrabee, 1946). The J receptors were identified as in the past (e.g. see Paintal & Anand, 1992) by noting that (1) the receptors were stimulated within 2·5 s of injections of about 100 μg PDG into the right atrium, (2) the receptors were not stimulated by a similar injection into the left atrium, and (3) the receptors were stimulated promptly (i.e. within 0·3 s) on insufflating halothane into the lungs.

All the physiological variables were initially recorded on a Racal DS 7 tape-recorder and subsequently photographed on continuously moving 70 mm photographic paper. From such records the maximum intensity of discharge, expressed as impulses per second, was measured by counting the maximum number of impulses that appeared within 1 s of injecting PDG or lobeline.

Drugs

Lobeline-HCl (Sigma, USA) was used. This was injected into the right or left atrium at a concentration of 100 μg ml^{-1} or higher. White crystalline powder of L-phenyl diguanide (Koch-Light Laboratories Ltd, Colnbrook, Bucks, UK) was also injected in the same concentration.

Observations on man

Approval by Ethical Committee. Lobeline-HCl is listed in the Indian Pharmacopaeia for intramuscular injection. Permission for injecting lobeline intravenously in much smaller doses into human volunteers was obtained from an Ethical Committee appointed by the Institute.

Subjects. Observations were made on twenty-six male volunteers (non-smokers). Their age ranged from 13 to 37 years (mean ± S.E.M., 24 ± 1·3 years) and their weight ranged from

38 to 70 kg (mean ± S.E.M., 53·0 ± 1·3 kg) Their (or their parents') informed consent was obtained. Each volunteer was informed that injections of lobeline or a placebo (normal saline) would be given intravenously. He came the next day and, after resting for about half an hour, he sat on a chair with an arm-rest on which his right arm was positioned.

Injection set-up. An indwelling cannula (Venflon) No. 20 gauge with a side-port was inserted into the right antecubital vein. It was connected to a saline drip via a short piece of rubber tubing with a clamp. Graded doses of lobeline-HCl at a concentration of 2 mg ml^{-1} were injected with a tuberculin syringe (or a 1 ml insulin syringe) through the side-port after clamping the rubber tubing. The clamp was released immediately after the injection so as to flush out the drug. The placebo (an equivalent volume of saline) was given in the same way. The interval between injections of lobeline was 10 min. The moment of flushing the cannula with saline was signalled with a foot switch. Most of the injections were given during or just before inspiration.

Signalling of sensations. The subject held a switch in his hand. He was told to press it when he felt any sensation in his throat or chest. About 1–2 min after the injection he was asked what he experienced. His statement was recorded verbatim in Hindi and translated into English. No subject was aware of what other subjects had reported. The lowest dose of lobeline, expressed as micrograms per kilogram, at which some sensations were felt was called the threshold dose for sensations. This threshold dose was determined by varying the doses injected at this level by ±0·1 mg through three or four injections. The errors involved in estimating the threshold dose for sensations in this way may have amounted to about 10%. Having estimated the threshold dose for sensations, the subsequent doses were increased in steps of 0·2 mg until the subject coughed. This dose was called the cough threshold dose. Each subject received about fifteen to twenty injections of lobeline. The interval between injections was 10 min.

Citric acid aerosol. Citric acid aerosol (0·5–2%) was generated with a nebulizer (Indian Oxygen Ltd, New Delhi) and was administered to the subjects in the usual way with a connecting tube. Local anaesthesia of the airways was achieved by making the subjects breathe an aerosol of 4% xylocaine (Astra IDL, Bangalore, India) generated by a De Vilbis ultrasonic nebulizer.

Record of respiratory movements. The respiratory movements were recorded with a stethograph placed around the chest of the subject. This was used for recording the pattern of respiratory movements. No attempt was made to record the tidal volume quantitatively, as we were only interested in recording a change in the pattern of breathing.

Recording set-up

The pneumogram and the timing signals for injection and sensation and the ECG (lead II) in some subjects were recorded either on a Beckman dynograph or on a Racal 4DS tape-recorder. In the latter case the records were displayed on an oscilloscope and photographed with a camera on 70 mm width continuously moving photographic paper. Since chopped amplifiers were used non-alignment of the several traces was eliminated.

Statistical analysis

Student's paired t test was used for comparing the responses of the same receptors to two drugs or the latencies of two responses (e.g. respiratory reflex and sensation latencies) in the same subject and deriving the significance of the differences from standard tables of P values (two tailed).

RESULTS

Effect of lobeline on RARs of cats

The effects of injecting lobeline into the right or left atrium were observed on seventeen RARs with adaptation indices ranging from 80 to 100%; in ten of them the adaptation index was 100%. The conduction velocities of their afferent fibres ranged from 13·0 to 37·6 m s^{-1}, which is typical of such fibres in the cat (Paintal, 1953). Since the cats were

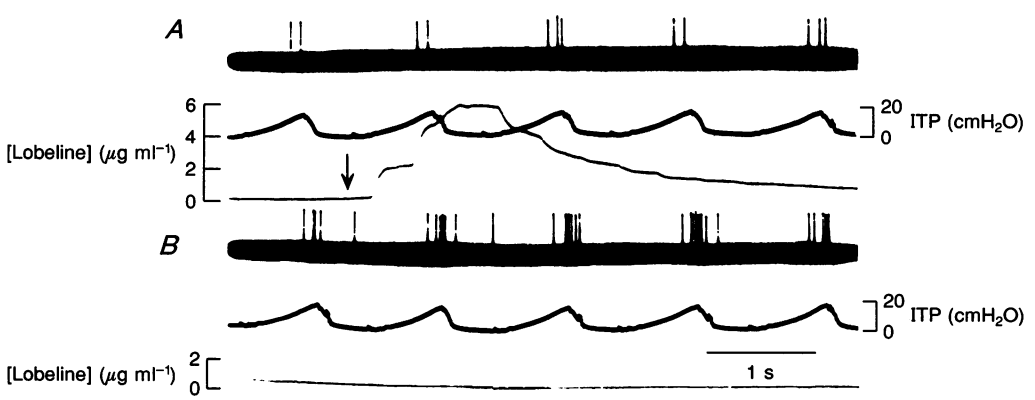

Figure 1. Effect of lobeline on an RAR

Conduction velocity of fibre, 24·4 m s^{-1}. Top trace, RARs; middle trace, intratracheal pressure (ITP); bottom trace, lobeline concentration. At arrow in A, 50 μg kg^{-1} lobeline was injected into the right atrium. This yielded a peak lobeline concentration of 5·5 μg ml^{-1} in the blood of the pulmonary artery. Sensitization of the ending started in B (continuation of record A) and built up with each subsequent respiratory cycle, as shown by the intratracheal pressure record (ITP, middle trace).

artificially ventilated the activity in the RARs consisted mainly of a burst of impulses during the inflation phase of the respiratory cycle, with far fewer or occasional impulses during the expiratory phase (Figs 1, 2 and 3).

The responses of seventeen such RARs from twelve cats to injections of 22 to 67 μg kg^{-1} lobeline into the right atrium were recorded; in fifteen of them the effect of similar injections into the left atrium was also recorded. The concentration of lobeline in the pulmonary artery was recorded in the case of ten receptors (e.g. see Figs 1 and 2). One receptor was unaffected by injecting 67 μg kg^{-1} lobeline into the right atrium. Three exceptionally sensitive receptors of one cat were stimulated, i.e. the discharge occurred in the absence of the natural stimulus (see Paintal, 1977). The remaining thirteen were sensitized, i.e. the excitatory effect appeared as an enhancement of the normally present activity during the inflation phase of the respiratory cycle without any change in intratracheal pressure in any cat after lobeline. This manifested itself as (1) a lowering of the threshold for excitation of the receptor by inflation, resulting in the onset of the inflation-linked burst at a lower intratracheal pressure, and (2) a highly significant ($P < 0{\cdot}01$) increase in the maximum intensity of discharge (Figs 1–3) from a control mean value of $3{\cdot}8 \pm 6{\cdot}9$ impulses s^{-1} (mean ± S.D.) to $32{\cdot}3 \pm 38{\cdot}7$ impulses s^{-1}.

The excitation did not set in during the expiratory phase in the receptors that were normally silent during expiration. This suggested that no impulses would be produced in these receptors by lobeline if it was injected during a prolonged expiratory period, such as the pause caused by stopping the respiratory pump. This was confirmed in the case of three RARs in three cats. In these the excitatory effect set in 11–15 s after the injection, i.e. only after the respiratory pump had been restarted and the intratracheal pressure had risen close to the excitatory threshold of the receptor. On the other hand, in the case of three exceptionally sensitive receptors in one cat, stimulation appeared after injecting lobeline while the respiratory pump was stopped. However, the excitation was less than that generated during normal inflation of the lungs, as expected. The preceding observations indicate that if lobeline is injected while artificial respiration is suspended (equivalent to breath holding in man), the output from the RARs will be much less than that produced by injecting the same dose of lobeline during normal respiration (compare activity in Fig. 1*B* with that in Fig. 2*B*). This conclusion is of

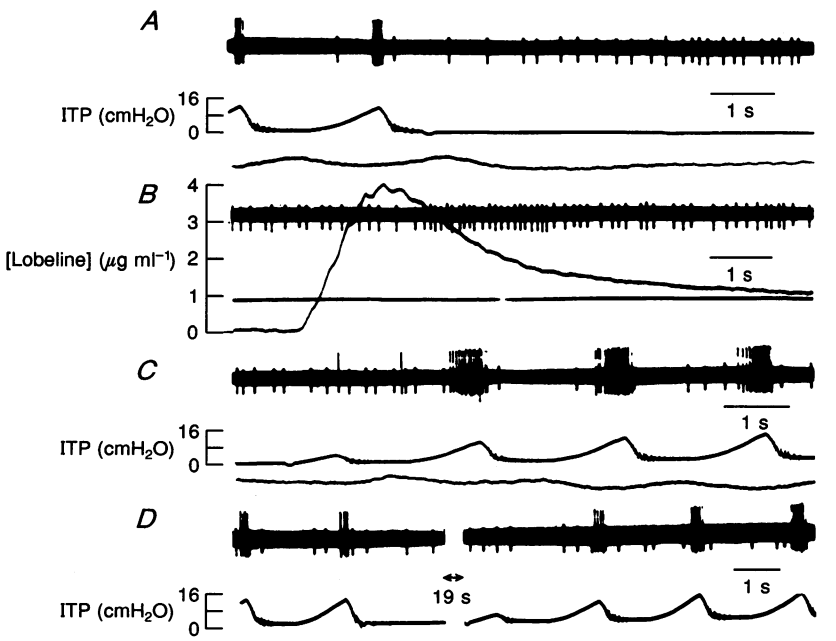

Figure 2. Effect of injecting lobeline on an RAR while artificial respiration was suspended
Conduction velocity of fibre, 20 m s^{-1}. Top traces, RARs; middle traces, ITP; bottom traces (*A–C*), lobeline concentration. *A* shows the normal activity in the RAR (large spikes) during each inflation by the respiratory pump. The pump was stopped in *A* (ITP, 0 cmH_2O) and 17 μg kg^{-1} lobeline was injected at the end of *A*. The concentration of lobeline rose to about 4 μg ml^{-1} in *B* but there was no increase in activity until the respiratory pump was restarted in *C* when marked sensitization set in after the concentration had fallen to 0 μg ml^{-1}. *D*, which is a control record, shows that repeating the procedure without injecting lobeline did not enhance the excitation of the receptor obviously over the control level of 5 impulses per cycle on restarting the respiratory pump after stopping it for 19 s. The small spiked impulses are from another fibre (unidentified). The bottom traces in *A* and *C* showing lobeline concentration and the middle trace in *B* showing ITP have no calibration bars.

relevance to observations during breath holding in man (see below).

Six RARs had one or more impulses during the deflation phase of the respiratory cycle produced by the respiratory pump. In all six of them this activity during the deflation phase increased to three to eight times the control values after injecting lobeline, as shown in Fig. 3*B*. As in the case shown in Fig. 2, this receptor was no longer excited when lobeline was injected after stopping the pump. In two receptors in two cats the effect of deflation produced by suction of 20–30 ml of air from the lungs, performed as described by Knowlton & Larrabee (1946) and Widdicombe (1954), was recorded before and after injecting lobeline. In both receptors the activity during suction of air after lobeline injection was about seven times greater than the mean activity (i.e. total number of impulses or duration of activity) produced by suction of air without lobeline. Figure 3*C* and *D* shows the responses of one of the receptors. The control response during suction shown in Fig. 3*C* was the largest of three control responses, one of which did not produce any impulse during suction. The observations showing the enhancement of responses during deflation after injecting lobeline are of relevance in connection with observations during forced expiration to residual volume after injecting lobeline in man (see below), even though the large changes in pressure recorded (Fig. 3*C* and *D*) are unlikely to occur in man when the chest is intact.

Latency for excitation

With the respiratory pump operating at higher speeds (e.g. Fig. 1) it was found that the latency for excitation by lobeline, which depended on the dose, varied from 1·4 to 6·3 s in different receptors following its injection into the right atrium. Twelve out of fifteen receptors tested (i.e. 80%) were unaffected by injections of the same dose of lobeline into the left atrium. Thus these receptors were accessible to lobeline only through the pulmonary circulation and not through the bronchial circulation. These results are in agreement with those reported earlier by Sant'Ambrogio & Sant'Ambrogio (1982). One of the receptors was excited with a smaller latency when lobeline was injected into the right atrium than when it was injected into the left atrium, thus suggesting accessibility through both circulations. Only one of the fifteen receptors was excited with a clearly shorter latency (1·8 s) after injection of lobeline into the left atrium than after injection of the same dose into the right atrium (3·7 s), thus indicating that this receptor was accessible through the systemic circulation.

Effect of lobeline on slowly adapting stretch receptors (SARs)

The effect of lobeline was tested on seven SARs in five cats. Six of them were unaffected by lobeline in doses that sensitized the RARs. The seventh receptor was clearly sensitized by injecting 67 μg kg^{-1} lobeline into the right atrium (latency, 2·9 s) but not by injecting the same dose into the left atrium, thereby indicating that it was accessible through the pulmonary circulation and not the bronchial circulation. This exceptional receptor was found in the same cat that had the three exceptionally lobeline-sensitive RARs described above.

Effect of lobeline on J receptors

As reported earlier (Paintal, 1971), it was confirmed that lobeline stimulated the J receptors but their responses to lobeline were much weaker than those to phenyl diguanide (PDG). The responses of seven receptors to both substances

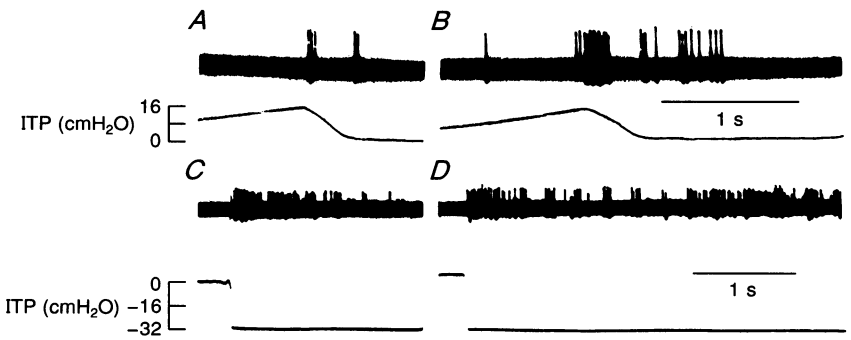

Figure 3. Effect of lobeline on the activity produced in two RARs during deflation

A is the control response in one receptor (fibre conduction velocity, 23 m s^{-1}) showing two impulses during the deflation phase of the respiratory cycle. *B* shows the greatly enhanced response during the deflation phase (as well as during the inflation phase) 20 s after injecting 28 μg kg^{-1} lobeline into the right atrium. *C* shows the maximum control response of another receptor (conduction velocity also 23 m s^{-1}) during suction of 30 ml air from the lungs. *D* shows the response of the same receptor to suction of air 23 s after injection of 45 μg kg^{-1} lobeline into the right atrium. The lower traces in each record are of intratracheal pressure (ITP).

Table 1. Threshold doses of lobeline and latencies for respiratory reflexes and sensations

Item	Dose of lobeline		Latency for resp. reflex		Latency for sensations		Difference between a and b (s)
	Range (μg kg^{-1})	Mean ± S.E.M. (μg kg^{-1})	Range (s)	Mean ± S.E.M.[a] (s)	Range (s)	Mean ± S.E.M.[b] (s)	
Sensation threshold	6·7–20·0	12·0 ± 0·7	6·5–16·8	10·6 ± 0·5*	6·2–15·2	11·0 ± 0·5*	0·4
Cough threshold	9·6–52·1	24·3 ± 2·5	6·1–16·4	9·1 ± 0·6**	6·0–16·0	9·4 ± 0·5**	0·3

*, ** Difference between the two respective means is highly significant ($P < 0·01$).

were compared. The maximum intensity of discharge measured over 1 s produced by about 46 μg kg^{-1} lobeline averaged 3·0 ± 0·9 impulses s^{-1} (mean ± S.D.). This was significantly greater ($P < 0·01$) than the control discharge of about 0·1 impulse s^{-1} but was significantly less ($P < 0·05$) than that produced by the same or a lower dose of PDG (mean ± S.D., 11·0 ± 3·5 impulses s^{-1}). However, in spite of this weaker response the mean latency for stimulation by lobeline, which averaged 1·9 ± 0·7 s, was significantly less ($P < 0·05$) than that following PDG (mean ± S.D., 3·1 ± 1·2 s). These shorter latencies (measured from injection signal) are reminiscent of those following nicotine (Paintal, 1955). Unlike PDG, lobeline showed evidence of tachyphylaxis, which was apparent in the case of three receptors in which the responses to a second or subsequent dose of lobeline were ineffective in stimulating the receptors for about 20 min. The responses returned thereafter. The other ten receptors studied did not show evidence of tachyphylaxis. The interval between injections was about 7 min.

Sensations produced in human subjects by lobeline

The mean threshold dose for sensations in twenty-six subjects was 12·0 ± 0·7 μg kg^{-1} (mean ± S.E.M.; Table 1). The shortest latency for sensations recorded in the three to four trials at the threshold dose was taken as the minimum latency at the threshold dose for each subject, assuming that the longer latencies in the other trials may have been due to unfamiliarity or to lower levels of alertness and longer reaction times. The mean latency for sensations was 11·0 s. The threshold dose for sensations remained constant after repeated injections (of even higher doses of lobeline), usually fifteen to twenty doses in each subject. Thus no evidence for tachyphylaxis was found. It is noteworthy that there were no false positive responses.

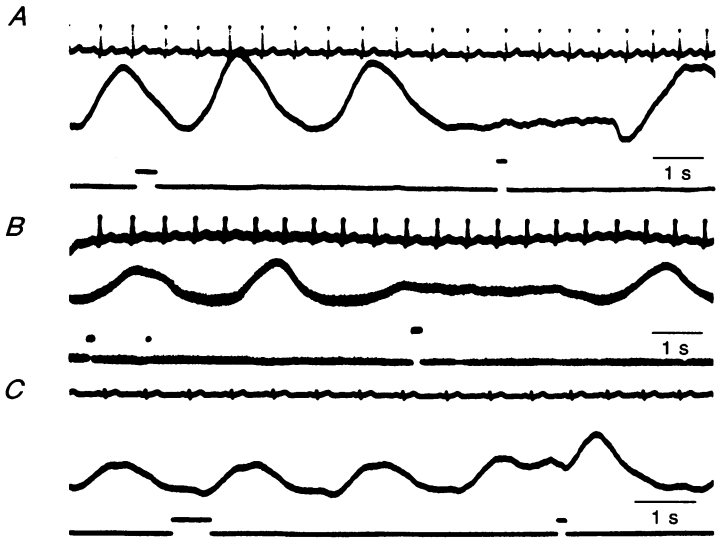

Figure 4. Effect of lobeline on two human subjects

Lobeline was injected at two different doses (18 μg kg^{-1} in *A* and 15 μg kg^{-1} in *B*) in one subject. Record *C* was obtained from another subject into whom 13·3 μg kg^{-1} was injected. The traces in each record are of ECG (lead II; top trace), pneumogram (inspiration upwards; middle trace); the bottom trace shows the injection signal followed by the sensation signal. In *A*, apnoea occured in expiration; in *B*, apnoea occurred in mid-inspiration (note variation in the same subject) and in *C* there was reflex prolongation of inspiration followed by a small increase in heart rate from 88 to 102 beats min^{-1}.

Table 2. Number of subjects (out of a total of 26) reporting specific sensations after lobeline injections

Type of sensation experienced	Location of sensations: Throat (no. of subjects)	Location of sensations: Upper Chest (no. of subjects)
Choking	11	0
Pressure	7	7
Smoke, fumes	5	4
Pain, burning	3	1
Tickling, minty air	3	0

The dose of lobeline was increased in a stepwise manner until the subject coughed after an injection. This dose was taken as the threshold dose for cough and in twenty-three subjects (three subjects requested termination of the test before completion) the mean threshold dose for cough was 24·3 μg kg^{-1}. At this dose the sensations were reported a little earlier by the subjects, the mean latency being 9·4 s, i.e. 1·6 s earlier than at the threshold dose for sensations (Table 1). (Latencies at other doses, even though shorter (e.g. Fig. 5*B*) than the latency at the cough threshold dose were not included in Table 1.) This is to be expected, as the latency will fall with increased concentrations of lobeline. Moreover the reaction time will be smaller at the higher intensities of sensations. The cough took longer to appear than the sensations (mean latency ± S.D., 11·1 ± 0·7 s; range, 7·3–24·0). The difference between the time for sensation perception and cough was 1·7 s. In four subjects the cough and sensation latencies were identical.

Reflex respiratory effects of lobeline

The sensation signals were nearly always accompanied by a reflex change in the pattern of breathing (see Jain *et al.* 1972). In most cases (44%) the reflex consisted of apnoea, i.e. prolongation of the expiratory pause (Fig. 4*A*). In 21% this prolongation was greater than 2 times the duration of expiration (T_e) (Fig. 3*A*) and in 23% it was less than 2 T_e. Prolongation of inspiration (Fig. 4*B* and *C*) was seen in 17%. The other less frequent reflex changes consisted of interruption of inspiration by expiration (Fig. 5*B*) in 14% and the reverse in 18%. In 11% a brief period of tachypnoea (Fig. 5*A*) occured without any preceding change in the respiratory pattern. The variations in the reflex effects described above were somewhat similar to those reported earlier in cats following stimulation of J receptors by PDG (Anand & Paintal, 1980). The cough, when it appeared, was superimposed on the initial reflex change. Usually the cough was preceded by an inspiratory effort; in a few instances the cough appeared during apnoea in expiration.

The mean latency for the appearance of the respiratory reflex at threshold dose for sensations was 10·6 s, i.e. 0·4 s earlier than the sensation (Table 1). At the higher doses, i.e. cough threshold dose, the mean latency for the respiratory reflex was 9·1 s, i.e. 0·3 s before the sensation. These observations suggest that the change in respiratory pattern (reflex) was not due to cortical influence arising from the sensation experienced.

In nine subjects the difference between the latencies for sensation and the reflex was 0·0–0·1 s (e.g. Fig. 4*B*).

Reflex bradycardia as reported by Bevan & Murray (1963) was seen infrequently, probably because the doses used in

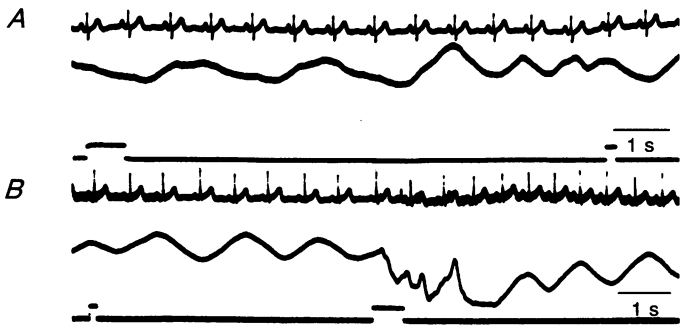

Figure 5. Records showing effect of lobeline in two subjects

Lobeline injected at doses of 12·5 μg kg^{-1} in *A* and 10 μg kg^{-1} in *B*. The middle trace in both records is the pneumogram (inspiration upwards). The first signal in the lowest trace of both records is the injection signal followed by the sensation signal. In *A*, three rapid shallow breaths set in just before the sensation was felt. In *B*, inspiration was interrupted by expiration just after the sensation.

the present investigation were relatively small, about a third of those used by Bevan & Murray (1963).

Nature and location of sensations

The subjects reported a variety of sensations. These were mainly choking, pressure or feeling of smoke in the throat and upper chest. However every subject reported the same sensation after each injection of lobeline at the threshold dose. Increasing the dose increased the intensity of the sensation, leading to some qualitative change as well as spread of sensation. Table 2 summarizes the sensations felt by the subjects. All twenty-six subjects reported sensations in the throat and twelve also reported sensations in the upper chest; six subjects reported two different sensations in the same area (throat or chest). The sensations at threshold doses as reported by them in Hindi are given in the Appendix along with the English translations and the roman Hindi equivalents.

Effect of injecting lobeline during breath holding

Since the activity of the RARs would be expected to be reduced while the breath is held (e.g. Fig. 2), attempts were made to see whether this reduced activity influenced the threshold dose of lobeline for producing sensations and coughing. In five subjects lobeline was therefore injected just before the breath was held at functional residual capacity (FRC). In four of them there was no change in the dose of lobeline needed for producing either sensation or coughing. In one subject the threshold dose for sensation increased from 12·5 to 15·6 μg kg^{-1}. Thus, taken as a whole, it can be concluded that breath holding did not produce any significant change in the threshold doses for sensations or coughing.

Effect of forced expiration to residual volume (RV) on responses to lobeline

After lobeline had been injected, five subjects were asked to breathe out quickly from FRC level to residual volume just before the sensations were expected to appear. This was done three times in each subject to see whether an input from RARs enhanced by lobeline (see Fig. 3*D*) would intensify the lobeline-induced sensations, thereby reducing the threshold doses needed for producing the sensations. However, this presumed enhanced activity in the RARs did not reduce the threshold dose of lobeline for sensations in any of the five subjects who carried out this manoeuvre. In fact the opposite, i.e. a significant ($P < 0·05$) increase in the threshold dose from a control value of 11·0 $\pm$ 4·2 μg kg^{-1} to 21·1 $\pm$ 7·1 μg kg^{-1} (mean $\pm$ S.D.) was observed in the five subjects. The reasons for this are currently being investigated.

Effect of deep inspiration on the responses to lobeline

The effect of a deep inspiration at the expected time of stimulation of the receptors by lobeline (i.e. just before the sensation signal) was examined on the same five subjects. In none of them was there any reduction in the sensation threshold dose of lobeline, thus indicating that an enhanced discharge in SARs or RARs did not facilitate the sensations produced by lobeline. On the other hand, as in the case of forced expiration, the threshold dose of lobeline for sensations was increased from a control mean value of 13·3 $\pm$ 3·7 μg kg^{-1} to 21·9 $\pm$ 8·3 μg kg^{-1} (mean $\pm$ S.D.) in five subjects. This increase was significant ($P < 0·05$).

Effect of expiration to RV without lobeline

None of the subjects reported any sensation while performing a forced expiration from FRC.

Sensations produced by citric acid aerosol

Five subjects were asked to inhale 0·5–2·0% citric acid aerosol generated by a nebulizer and report the sensations that they felt. They reported a feeling of irritation in the throat leading to coughing but they did not feel any of the sensations which they had experienced after injections of lobeline. Injecting lobeline while the subjects breathed citric acid aerosol did not alter the threshold dose for producing the lobeline-induced sensations. In five subjects the cough produced by citric acid aerosol was blocked by making the subjects breathe xylocaine aerosol (4%). Injecting lobeline after such a block did not alter the dose at which the sensation and coughing were produced.

DISCUSSION

It has been shown that the SARs of man respond in qualitatively the same way as those of cats and dogs (Langrehr, 1964; Guz & Trenchard, 1971). Such similarity of behaviour has not been shown in the case of the RARs. However, the RARs of monkeys respond in the same way as those of cats (Zucker & Gilmore, 1977). In fact Zucker & Gilmore found that, as in cats, out of a total 347 pulmonary afferent fibres which they isolated from the vagus of the monkey, only about 10% were RARs and these receptors responded in the same way as the RARs of cats to large inflations of the lungs, as described by Knowlton & Larrabee earlier (I. H. Zucker, personal communication, 1991). It can therefore be assumed that RARs also exist in man and that they respond in qualitatively the same way as those of cats and dogs to natural stimuli, i.e. inflation and deflation.

From the above it follows that forced expiration from FRC level should produce a high-frequency burst of impulses in the RARs of man without stimulating the SARs, since deflation is known to produce such a burst in cats (Knowlton & Larrabee, 1946; Widdicombe, 1954). However, the present results show that if a barrage of impulses from the RARs is produced in man by forced expiration the sensations in the throat or chest are not produced, as no sensations similar to the lobeline-induced sensations were experienced by any one of the subjects who carried out this manoeuvre several times.

The lobeline-induced sensations could be produced by SARs, RARs, J receptors, or some other unidentified receptors that are specifically stimulated by lobeline. There is no evidence for stimulation of the RARs of man by lobeline. Nevertheless in the present study we assumed, to start with, that lobeline does excite the RARs and thereafter we proceeded to determine whether the presumed excitation of the RARs by lobeline could account for the lobeline-induced sensations experienced by the subjects. We also assumed that the pattern of excitation consisting of sensitization is similar to that observed in the RARs of cats (Figs 1–3). In cats lobeline greatly enhances the responses of the RARs to the natural stimulus, i.e. it sensitizes the receptors (Figs 1–3) to both inflation and deflation. If the same occurs in man then the burst of impulses produced by forced expiration (see Fig. 3*D*) should be enhanced by a prior appropriately timed injection of lobeline and forced expiration. However, the present observations have shown that such impulses, even if greatly enhanced by lobeline, did not enhance the lobeline-induced sensations, since the threshold dose of lobeline needed for producing the sensations or coughing was not reduced by forced expiration at the appropriate time in any one of the subjects. Similarly there was no reduction in the threshold dose for sensations or coughing when a deep breath was taken at the time when the sensations were expected. A deep inspiration would be expected to yield a high intensity burst of impulses not only in the SARs but also in the RARs, as judged by the responses of the RARs of cats to inflation (Knowlton & Larrabee, 1946; Widdicombe, 1954; present results, e.g. Figs 1 and 2). Consistent with these observations is the fact that injecting lobeline while the breath was held (i.e. at a time when the activity of the RARs would be reduced or absent; see Fig. 2) did not raise the threshold dose needed for producing the sensations.

The above results indicate that inputs from SARs and RARs do not seem to be primarily involved in the production or enhancement of the lobeline-induced sensations. This conclusion is consistent with the fact that injection of lobeline into the left ventricle of man, which would stimulate all RARs accessible through the systemic circulation, does not produce a dry cough (Stern *et al.* 1966). The same observation also excludes the bronchial C fibre receptors (Coleridge & Coleridge, 1977) from playing any role in the production of the lobeline-induced sensations. Thus since all the above receptors do not seem to play an obvious role it follows that the J receptors are most probably responsible for the sensations. Two main sources of evidence, a report by Bevan & Murray (1963) and a later report by Jain *et al.* (1972), indicate that lobeline stimulates the J receptors of man. Bevan & Murray showed that ventilatory depression, bradycardia and hypotension (a response attributable to J receptor stimulation in animals; see Paintal, 1973; Coleridge & Coleridge, 1984) appeared on injecting 30–80 μg kg^{-1} lobeline intravenously in man. They concluded that their observations constituted strong evidence for a ventilation-regulating reflexogenic area between the large veins and the pulmonary circulation. Subsequently Jain *et al.* (1972) were able to delimit the reflexogenic area to the receptors in the lungs between the pulmonary artery and the left atrium by observing that reflex apnoea occurred on injecting lobeline into the pulmonary artery. They therefore concluded that the reflex respiratory effects produced within 2 s of injections of lobeline into the pulmonary artery of man must be due to stimulation of the J receptors – a conclusion that is generally accepted (e.g. see pp. 39 and 71 in Coleridge & Colderidge, 1984). The fact that injecting lobeline into a distal branch of the pulmonary artery does not elicit coughing (Stern *et al.* 1966) can be attributed to the J receptors being essentially bypassed. This has been shown to occur in cats when capsaicin is injected into a distal branch of the pulmonary artery resulting in the absence of the typical pulmonary chemoreflex (Pórsász, Such & Pórsász-Gibiszer, 1957; see also Paintal, 1986*b*). Lobeline is so far the only drug known to produce the pulmonary chemoreflex in man; neither PDG nor capsaicin produce the reflex effects produced by lobeline and so neither of them produce the kinds of sensations produced by lobeline (Jain *et al.* 1972; Winning, Hamilton, Shea & Guz, 1986).

Certain important differences between the sensations produced by capsaicin and lobeline are noteworthy. For example, capsaicin produced retrosternal burning at the threshold dose for producing any sensation whatever. Coughing appeared in one out of two subjects on injecting a higher of dose of capsaicin. The retrosternal burning sensation was abolished by prior local anaesthesia of the airways with a local anaesthetic aerosol (Winning *et al.* 1986). On the other hand, as shown by the present results,

lobeline at threshold doses never produced pain but it gave rise to certain characteristic sensations (Table 2 and Appendix). Coughing appeared in all subjects when the dose was approximately doubled (Table 1). Pain or a burning sensation was felt by only 12% of the subjects at the higher doses. Finally, none of the sensations or coughing was blocked by prior anaesthesia of the airways with a local anaesthetic aerosol. These differences suggest that two different sets of receptors in two different locations are involved in producing the sensations following injections of lobeline and capsaicin.

Table 1 shows that the sensations appeared barely 0·3 s after the onset of the respiratory reflex effects when the dose of lobeline injected was twice the threshold dose for producing the sensations only. This interval can be accounted for by the reaction time between the subject experiencing the sensations and pressing the switch. It is noteworthy that in nine subjects the difference between the latencies for sensation and the reflex was 0·0–0·1 s, which indicates that the reflex effects and sensations are probably produced by the same sensory mechanism. Therefore since the respiratory reflex effects are produced by the J receptors (Jain *et al.* 1972) it follows that the sensations can also be attributed to them. In this connection it is worth noting that the sensations are not affected by simultaneously present sensations in the throat, e.g. sensations of irritation generated by citric acid aerosol. Moreover, unlike the block of the capsaicin (I.V.)-induced burning sensation in the chest by local anaesthesia of the airways (Winning *et al.* 1986), the lobeline-induced sensations are not blocked by local anaesthesia of the airways with anaesthetic aerosols.

Finally only 12% of the subjects reported pain or burning (Table 2). This result contrasts with the report of substernal burning in six out of seven subjects tested by Eckenhoff & Comroe (1951). The difference can be attributed to the fact that the dose used by Eckenhoff & Comroe was much higher than ours, 5–7·5 mg as compared to < 2 mg in the present investigation. It can be assumed that the sensation of burning pain that occurs at higher doses arises from the same mechanism that produces the sensations of choking, pressure, feelings of fumes, smoke, or gas in the throat and chest. If the sensations are indeed due to impulses from the J receptors then this conclusion is likely to be of clinical importance, especially in cases of interstitial oedema which stimulates the J receptors markedly (Paintal, 1969; Coleridge & Coleridge, 1977; Roberts, Bhattacharya, Schultz, Coleridge & Coleridge, 1986). Here it is pertinent to note that pain was reported by 72% of the thirty-six normal young soldiers who developed high-altitude pulmonary oedema within 12–96 h of being transported by air to a height of 3200 m (Paintal, 1986*a*). It is not known whether these soldiers had sensations in the throat and chest other than pain, but most of them certainly had a dry cough, which accompanies the sensations when the stimulation of J receptors is sufficiently intense, as observed in the present investigation.

The inevitable conclusion that follows from the above is that stimulation of the J receptors produces coughing in man as a consequence of intensification of certain sensations in the throat. At first sight this conclusion may appear to be inconsistent with the observations of Tatar, Webber & Widdicombe (1988) showing that relatively intense stimulation of the J receptors with 25–50 μg kg^{-1} phenyl biguanide inhibits coughing generated by mechanical stimulation of receptors in the larynx and trachea. In fact there is no inconsistency because since impulses from J receptors inhibit all somatic muscles (Paintal, 1970; Deshpande & Devanandan, 1970; Schiemann & Schomburg, 1972) it is not surprising that the muscles that produce coughing on mechanical irritation of the trachea are also inhibited by stimulating the J receptors, as observed by Tatar *et al.* (1988). In fact their observations are likely to be clinically important, as they suggest that the cough reflex may be depressed in conditions in which the J receptors are stimulated relatively intensely, e.g. in severe pulmonary congestion.

APPENDIX

Sensations experienced by the subjects after injection of lobeline, as reported by them in Hindi (Roman Hindi in parentheses) along with English translation:

1. **गले में दबाव और घुटन रूक रूक कर होता है** (Gale mein dabav aur ghutan ruk-ruk kar hota hai)	Intermittent pressure and choking (or suffocation) in throat
2. **छाती से ऊपर उठता हुआ हवा का दबाव** (Chhati se upar uthta hua hawa ka dabav)	Pressure of wind coming upwards from chest
3. **गले को खींचता हुआ छाती में दबाव** (Gale ko khinchta hua chhati mein dabav)	Pressure in chest pulling the throat
4. **छाती से ऊपर उठता हुआ गले में दबाव** (Chhati se upar uthta hua gale main dabav)	Pressure in throat rising up from chest
5–6. **गले में दबाव और घुटन** (Gale mein dabav aur ghutan)	Pressure and choking (or suffocation) in throat
7. **दम घुटता है और साथ में छाती में दबाव** (Dam ghutata hai aur saath mein chhati mein dabav)	Suffocation and pressure in chest
8. **गले में धुआँ आता हुआ दम घोटता है** (Gale mein dhuan aata hua dam ghotata hai)	Smoke coming in throat and suffocating
9. **हवा गले में गुदगुदी करती है और साँस रोकती है** (Hawa gale mein gudgudi karti hai aur saans rokti hai)	Air tickling the throat and stopping breath
10. **धुआँ उमड़ता हुआ छाती से गले में आकर घुटन करता** (Dhuan umadta hua chhati segale mein aakar ghutan karta)	Smoke gurgling in chest and rising to throat producing choking (or suffocation)
11. **छाती में भारीपन, खाँसी सी गले में अटकती है** (Chhati mein bhaaripan, khaansi see gale mein atakti hai)	Heaviness in chest, with 'cough' like thing stuck in throat
12. **छाती में धुआँ उठता हुआ, साँस लेने में मुश्किल** (Chhati mein dhuan uthta hua, saans lene mein mushkil)	Smoke rising in chest making breathing difficult
13. **गले में धुआँ बनता है और घुटन सी होती है** (Gale mein dhuan banta hai aur ghutan see hoti hai)	Smoke in throat and choking (or suffocation)
14. **गले में नीचे से गुदगुदी सी होती है** (Gale mein neeche se gud-gudi see hoti hai)	Tickling in throat from below
15. **गला घुटता है** (Gala ghutata hai)	Choking or suffocation in throat (although he points towards manubrium)
16. **छाती में गैस बनती है** (Chhati mein gas banti hai)	Gas in chest
17. **गैस गले में अटकती है** (Gas gale mein atakti hai)	Gas getting stuck in throat
18. **जलने का सा धुआँ गले में आता है** (Jalne ka sa dhuan gale mein aata hai)	Feeling burning fumes in throat
19. **छाती में दबाव बनता है** (Chhati mein dabav banta hai)	Pressure in chest
20. English-speaking medical student:	Sudden want of air; trachea constriction behind sternum; wave of cold minty air from manubrium up to Adam's apple pressing throat
21. **गले में धक सा होता है और छाती में दर्द** (Gale mein dhak sa hota hai aur chhati mein dard)	Presure in throat and pain in chest
22. **दम घुटा, गले में धक्का, बीड़ी का धुआँ** (Dam ghuta; gale mein dhakka; bidi ka dhuan)	Suffocation; pressure jolt in throat; feeling of bidi (cigarette) smoke
23. **छाती में धक्का गले में जाता हुआ मिर्च लगाता है** (Chhati mein dhakka, gale mein jata hua, mirch lagaata hai)	Pressure in chest, rising to throat, giving chilli-like sensation
24. **दबाव की लहर गले में घुटन करती हुई** (Dabav ki lehar gale mein ghutan karti hui)	Pressure waves in throat producing choking (or suffocation)
25. **गले में तेजाब का धुआँ कड़वाहट और दम घोटता हुआ** (Gale mein tejaab ka dhuan kadwahat aur dam ghotata hua)	Acid fumes producing bitterness and suffocation in throat
26. **छाती में खिंचाव और दम भारी होता है** (Chhati mein khinchav aur dam bhaari hota hai)	Constriction in chest with choking (or suffocation)

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