Effect of protriptyline on ventilatory responses to hypercapnia and asphyxia in normal subjects

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ABSTRACT: A double-blind crossover study was undertaken to assess the effect of protriptyline on ventilatory responses in normal subjects. Seven subjects received in random order placebo, 10 mg and 20 mg protriptyline daily for 2 weeks. Measurements of hypercapnic ventilatory response (HCVR) and asphyxial hypoxic ventilatory response (HVR) were made before treatment, 6-8 h after the first dose, and after 2 weeks treatment. Mean HCVR and HVR following 10 mg and 20 mg protriptyline did not differ significantly from measurements on placebo, neither for the single dose study or after 2 weeks.

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The stimulant tricyclic agent protriptyline decreases episodic nocturnal desaturation in patients with obstructive sleep apnoea [1,2] and more recently, has been shown to improve nocturnal gas exchange and diurnal arterial oxygen saturation in patients with sleep related disturbances of breathing secondary to restrictive chest wall disease [3] and chronic airflow limitation [4]. The mechanism of action of protriptyline in sleep disordered breathing has been attributed to a reduction in rapid eye movement (REM) sleep associated episodes of apnoea and hypopnoea, with an additional action on upper airway muscle tone [5]. However, an independent respiratory stimulant effect has not been excluded.

This trial was carried out to assess the effect of protriptyline on hypercapnic and hypoxic ventilatory drive in normal subjects. The response to a hypercapnic hypoxic (asphyxial) stimulus rather than to a conventional eucapnic hypoxic challenge was chosen for two reasons. Firstly, hypercapnia potentiates the effect of hypoxia on peripheral chemoreceptors [6] and secondly, as the drug is being used increasingly in patients with nocturnal hypoventilation, hypercapnic hypoxia would seem a more relevant stimulus.

Subjects and methods

The effect of protriptyline on hypercapnic ventilatory response (HCVR) and asphyxial hypoxic ventilatory response (HVR) was assessed in seven normal subjects (3 male; 4 female) aged 27-53 yrs (mean age 36.1 yrs). Subjects were hospital staff members with no previous experience of the measurement techniques. All were nonsmokers with no history of cardiovascular disorders and normal spirometry. The study was approved by the hospital Ethics Committee and informed consent was obtained from participants.

The double-blind, placebo-controlled, crossover trial consisted of 3 phases each lasting 2 weeks, with a 2 week washout period between each phase. In random order subjects received placebo, 10 mg protriptyline or 20 mg protriptyline daily for the duration of the phase. Measurements of HCVR and HVR were made the day before starting each phase, on the first day of each phase 6-8 h after ingestion of the tablet (acute study), and after 2 weeks treatment (long term study). After the first day of each phase subjects were requested to take the trial tablet on retiring. The timing of the studies of both acute and long term effects was selected because peak levels following a single oral dose of protriptyline are reached after 6-12 h and stable plasma levels are demonstrable in the majority of subjects after 2 weeks [7,8]. Half-life varies between subjects with a mean value estimated at 78.4 h [9].

The order of HCVR and HVR was randomised and measurements were made at the same time of day. The 4 week period between assessments meant the measurements were probably made at the same stage of the menstrual cycle in each female subject.

Venous samples for determination of protriptyline levels were collected at each measurement session with the exception of the first. Samples were analysed by gas liquid chromatography [10].

Assessment of ventilatory response

Measurements were made after subjects breathed at the mouthpiece for 5-10 min until end-tidal carbon dioxide tension (Pco_2) was stable. Ventilatory response
to hyperoxic hypercapnia was determined using a rebreathing technique [11]. Subjects rebreathed for 4 min from a 6 l reservoir bag containing 7% carbon dioxide in 93% oxygen. Expiratory flow was intergrated to give a volume trace using a Fleisch pneumotachograph (size 3) and end-tidal \( P_{CO_2} \), was sampled using a Hewlett Packard capnometer (model 47201A). The pneumotachograph and capnograph were calibrated before each experimental session with room air and 7% carbon dioxide in 93% oxygen. Calibrations were checked at the end of each session. Two sets of measurements of HCVR were made at each assessment and the mean of these values was used for analysis. To calculate HCVR, measurements during the first 45 s of rebreathing were discarded and ventilation over subsequent 30 s periods was plotted against the end-tidal \( P_{CO_2} \) value at the mid-point of each period. The ventilatory response to hypercapnia, expressed by the slope of this relationship, was calculated by linear regression analysis. The position of the response line was established by estimating ventilation at an end-tidal \( P_{CO_2} \) of 55 mmHg (7.3 kPa) and termed \( V_{\text{55}} \).

The ventilatory response to hypercapnic hypoxia was measured using the method of Hensley and Read [12]. A controlled decrease in arterial oxygen saturation to approximately 70%, in the presence of a stable elevation of \( P_{CO_2} \), was obtained by breathing from two reservoir bags in sequence. From breathing room air, the subject was switched into a one-way circuit and rebreathed for 4–5 breaths from a bag containing 7% carbon dioxide in air. Without the subject's awareness he was then switched to a second bag containing 7.5% carbon dioxide in nitrogen. The expiratory tap was open to air at this stage so that rebreathing did not occur. When arterial oxygen saturation began to fall the subject was switched back to the bag containing carbon dioxide in air and rebreathed this mixture until the ramp of desaturation was complete. Arterial oxygen saturation (\( SaO_2 \)) was monitored continuously using a Hewlett Packard oximeter model 47201A whose response to a step change in input from 100–80% is 90% complete in 1.3 s. The mean of three measurements of HVR was used for analysis. Ventilatory response to asphyxia was calculated by plotting breath by breath minute ventilation against arterial oxygen saturation at the start of each breath. No attempt was made to correct the arterial oxygen saturation reading for the instrument response time. The position of the HVR line was determined by calculating ventilation at a saturation level of 95% (\( V_{95} \)).

Regression lines were only accepted if the correlation coefficient exceeded 0.8. HCVR and HVR before and after placebo, 10 mg and 20 mg were compared by 2-way analysis of variance. Differences between male and female subjects were analysed using the Mann-Whitney test. The correlation between dose (mg·kg\(^{-1}\)) and plasma levels, and dose (mg·kg\(^{-1}\)) and side effects was calculated using the Spearman rank correlation test.

**Results**

HCVR, HVR, \( V_{55} \) and \( V_{95} \) on entry to the trial were greater in male subjects than females, although these differences were not significant (\( p>0.05 \)).

Values before and after placebo, 10 mg and 20 mg protriptyline are shown in figures 1–4. No placebo effect was evident and 10 mg and 20 mg protriptyline did not alter the slope or position of the response curve in both the acute and long term studies. No trend between response to 10 mg and 20 mg was observed.

Inter-subject variation in pre-treatment data is clearly shown, but no overall difference was seen comparing initial values for each phase. The coefficient of variation for control tests was 0.24 for HCVR and 0.37 for HVR.

The lower limit of accurate measurement of protriptyline using the gas liquid chromatographic method is 5 \( \mu g·l^{-1} \). Levels below 5 \( \mu g·l^{-1} \) are therefore denoted as undetectable and assumed to be zero. Plasma protriptyline levels are shown in table 1.

No correlation was found between dose (mg·kg\(^{-1}\)) and plasma levels. All subjects noted a dry mouth

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Fig. 1. — Hypercapnic ventilatory response (HCVR) before and after 2 weeks treatment with placebo, 10 mg and 20 mg protriptyline-day\(^{-1}\). Mean values and 95% confidence intervals (CI) of change in HCVR are shown.
HYPOXIC (ASPHYXIAL) VENTILATORY RESPONSE (HVR)

Fig. 2. - Hypoxic (asphyxial) ventilatory response (HVR) before and after 2 weeks treatment with placebo, 10 mg and 20 mg protriptyline-day⁻¹. Mean values and 95% confidence intervals (CI) of change in HVR are shown. $Sao_{2}$: arterial oxygen saturation.

HYPERCAPNIC VENTILATORY RESPONSE

Fig. 3. - Minute ventilation at end-tidal $PCO_{2}$ level of 55 mmHg ($\dot{V}_{E}$) before and after 2 weeks treatment with placebo, 10 mg and 20 mg protriptyline-day⁻¹. Mean values and 95% confidence intervals (CI) for change in $\dot{V}_{E}$ are shown.

HYPOXIC (ASPHYXIAL) VENTILATORY RESPONSE

Fig. 4. - Minute ventilation at arterial oxygen saturation level of 95% ($\dot{V}_{E}$) before and after 2 weeks treatment with placebo, 10 mg and 20 mg protriptyline-day⁻¹. Mean values and 95% confidence intervals (CI) for change in $\dot{V}_{E}$ are shown.
on active drug and in this respect it was difficult to blind the trial. Five subjects noted other anticholinergic side effects including constipation and mild visual disturbance.

Discussion

Protriptyline had no effect on the slope or position of hypercapnic and hypoxic ventilatory response in normal subjects. Several points are worth consideration. First, the pneumotachograph and capnometer were not calibrated with all the gas mixtures used to test HCVR and HVR. This will introduce small errors into the measurements of tidal volume and carbon dioxide. Since the experimental procedure was standardized, it is unlikely that these measurement errors will have biased the study against finding any change in ventilatory responsiveness due to protriptyline administration.

Second, inter-subject variation and variation within subjects over time [13] limits interpretation of ventilatory response data. Within subject coefficient of variation for control tests was 0.24 for HCVR and 0.37 for HVR, values similar to those reported previously using the same methods to induce hypercapnia and hypoxia [12-15]. It is possible that a slow ramp of isocapnic hypoxia such as that used in the method of Rebuff and Campbell [16] may have restricted variability of HVR. Our interest in the effects of protriptyline in obstructive sleep apnoea led us to choose a brief ramp of moderate asphyxia as the relevant stimulus. The baseline variability of HCVR and HVR may have hidden an effect of protriptyline that would be of pharmacological interest and that might have thrown new light on mechanisms controlling breathing. This was not the purpose of the study, however, which was directed at examining an effect of protriptyline on ventilatory control of sufficient magnitude to explain improvement of nocturnal oxygenation in a number of clinical settings. Given the 95% confidence intervals of the mean differences between control and drug studies, it is possible that mean HCVR increased from 1.73–2.28 l·min⁻¹·mmHg⁻¹ after two weeks administration of protriptyline 10 mg and from 1.75–2.47 l·min⁻¹·mmHg⁻¹ after 2 weeks of protriptyline 20 mg. Similarly, HVR may have increased from 0.83–0.96 l·min⁻¹·% Sao₂ after protriptyline 10 mg and from 1.39–2.10 l·min⁻¹·% Sao₂ after protriptyline 20 mg. The data are not consistent with a drug effect greater than this and, therefore, we consider it unlikely that an effect has been missed that would be clinically meaningful in the context of nocturnal hypoxaemia. Sleep is associated with an approximate halving of HCVR and to a lesser extent HVR [17-21]. If the effect of protriptyline on nocturnal hypoxaemia in obstructive sleep apnoea [1, 2], restrictive chest wall disease [3] and chronic airflow limitation [4] was due primarily to augmentation of ventilatory responsiveness the drug should restore sleeping responsiveness towards daytime values and thus it might be expected to double awake ventilatory response. We therefore chose conservatively to accept a 50% increase in awake HCVR and HVR as being clinically meaningful. The power of the study to detect such an increase in HCVR and HVR at the 0.05 level was 99% and 92% respectively.

A third consideration is that HCVR and HVR were not measured during sleep. It is unlikely that protriptyline has substantial effect on ventilatory control during sleep without clearly measurable effects awake but the point remains to be tested. It is relevant to note that prochlorperazine doubles HVR awake [15] and asleep [21] yet does not have any clinically significant effect on apnoea index or oxygenation in patients with obstructive sleep apnoea [21].

Plasma levels after 2 weeks treatment with 10 and 20 mg protriptyline were lower than those obtained after 6 weeks treatment with a similar dose in patients with restrictive chest wall disease [3], despite a greater mg·kg⁻¹ dosage in normal subjects receiving 20 mg. As many of the patients with restrictive chest wall disease were in cardiorespiratory failure and their mean age was greater, altered hepatic metabolism and plasma protein binding were likely to have contributed to differences in plasma concentration of protriptyline. Also, it should be noted that plasma levels were measured in the normal subjects after 2 weeks treatment, but at 4–6 wks in the restrictive chest wall disease group. One possible consequence of the low plasma concentrations is that a significant effect on HCVR and HVR was missed. However, if this was so a trend in response between 10 and 20 mg might be expected and this was not seen. The difficulty of establishing the presence of concentrations of less than 5 μg·l⁻¹ is a source of concern, but in the absence of a more discriminant assay, is an unavoidable source of error.
The conclusion that protriptyline has no effect on HCVR and HVR supports the work of others. Brownell et al. [1] found that protriptyline did not alter HCVR in normocapnic patients with obstructive sleep apnoea, and Conway et al. [22] were unable to demonstrate a change in eucapnic HVR in a similar group with sleep apnoea. The data are also consistent with that obtained from a trial of protriptyline in patients with restrictive chest wall disease [3]. In the latter study, no change in diurnal arterial Pco\textsubscript{2} or base excess was seen; an unlikely finding if the drug stimulates HCVR. Also drugs such as almitrine which enhance peripheral chemoreceptor drive tend to lower Pco\textsubscript{2} by increasing total ventilation [23].

Finally, if HVR is a mechanism for limiting nocturnal episodes of desaturation, a drug which stimulates HVR would be expected to promote earlier termination of apneic/hypopneic episodes, thereby reducing the duration of such events and minimum arterial oxygen saturation. Almitrine has been shown to reduce minimum arterial oxygen saturation and time spent at a level of oxygen saturation of less than 80% in patients with chronic bronchitis and emphysema [24]. In contrast, protriptyline had no effect on minimal arterial oxygen saturation in patients with restrictive chest wall disease. In these individuals the improvement in nocturnal oxygenation is closely correlated with a reduction in REM sleep related hypopneic episodes. We conclude that stimulation of HCVR and HVR is unlikely to be an additional clinically significant mechanism of action of protriptyline.

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References


Absence d'effet de la protriptyline sur les réponses ventilatoires à l'hypercapnie et à l'asphyxie chez les sujets normaux. A.K. Simonds, N. Carroll, M.A. Braithwaite, N.A. Saunders. RÉSUMÉ: Une étude en double aveugle avec perméation croisée a été entreprise pour déterminer l'effet de la protriptyline sur les réponses ventilatoires des sujets normaux. Pendant 2 semaines, le placebo et des doses de 10 et 20 mg de protriptyline ont été administrés quotidiennement chez 7 sujets de manière randomisée. Des mesures de la réponse ventilatoire à l'hypercapnie et de la réponse ventilatoire à l'asphyxie hypoxique ont été réalisées avant traitement et 6 à 8 h après la première dose ainsi qu'après 2 semaines de traitement. Les valeurs moyennes de la réponse ventilatoire hypercapnique et de la réponse ventilatoire à l'asphyxie hypoxique après 10 et 20 mg de protriptyline ne diffèrent pas significativement des mesures après placebo, que ce soit après une dose unique ou après 2 semaines. Eur Respir J, 1989, 2, 758–762.