Baclofen Destabilises Breathing during Sleep in Healthy Humans: A Randomised, Controlled, Double-blind Crossover Trial

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Title

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Principal investigator statement

The authors confirm that the principal investigator for this paper is Pr Christian STRAUS and that he had direct responsibility for the participants to the study.

Running head

Baclofen and breathing during sleep

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This report conforms with the CONSORT statement for reporting randomised trials (checklist provided).

What is already known about this subject

Periodic breathing is frequent in patients with severe heart failure. It is an indicator of severity. It also has its own deleterious consequences on sleep quality. This justifies attempts to correct it irrespective of the underlying disease. There is currently no effective pharmacological treatment of periodic breathing. Animal models and human data suggest that baclofen, a $GABA_B$ agonist used to treat spasticity and alcohol dependence, can reconfigure respiratory central pattern generators.

What this study adds

We hypothetised that baclofen could attenuate the breathing instability induced by hypoxia during sleep in normal humans. Contrary to this hypothesis, baclofen aggravated breathing instability during hypoxic sleep. Caution should be exerted when prescribing baclofen to patients with or at risk of sleep-disordered breathing.

Abstract

Aim. Periodic breathing is frequent in patients with severe heart failure. Apart from being an indicator of severity, periodic breathing has its own deleterious consequences (sleep-related oxygen desaturations, sleep fragmentation), which justifies attempts to correct it irrespective of the underlying disease. Animal models and human data suggest that baclofen can reconfigure respiratory central pattern generators. We hypothesised that baclofen, a $GABA_B$ agonist, may thus be able to correct periodic breathing in humans.

Methods. Healthy volunteers were exposed to hypoxia during sleep. Participants who developed periodic breathing (n=14 — 53 screened—) were randomly assigned to double-blind oral baclofen (progressively increased to 60 mg per day) or placebo.

Measurements.The primary outcome was the coefficient of variation of respiratory cycle total time (CoVarTT) considered as an indicator of breathing irregularity. Secondary outcomes included the coefficient of variation of tidal volume (CoVarVT), apnoea-hypopnoea index, sleep fragmentation index and ventilatory complexity (noise limit).

Results. The analysis was conducted in 9 subjects after exclusion of incomplete datasets. CoVarTT significantly increased with baclofen during non-REM sleep (median with placebo 56.00% [37.63 - 78.95]; baclofen 85.42% [68.37 - 86.40], p=0.020; significant difference during the N1-N2 phases of sleep but not during the N3 phase). CoVarVT significantly increased during N1-N2 sleep. The apnoea-hypopnoea index, sleep fragmentation index and ventilatory complexity were not significantly different between placebo and baclofen.

Conclusion. Baclofen did not stabilise breathing in our model. On the contrary, it increased respiratory variability. Baclofen should probably not be used in patients with or at risk of periodic breathing.

Introduction

Periodic breathing consists of clusters of breaths (with or without a crescendo-decrescendo pattern) separated by a period of central hypopnoea or apnoea. It is common during wakefulness and sleep in human neonates. Conversely, periodic breathing in adults generally reflects dysfunction of brainstem respiratory central pattern generators. It is frequent in severe heart failure, where it is a likely consequence of brainstem hypoperfusion and constitutes a negative prognostic indicator [1]. In addition to its prognostic value, periodic breathing has its own deleterious consequences, as it induces haemoglobin desaturation and disrupts sleep, which justifies efforts to correct it independently of its causative mechanisms. Periodic breathing can be corrected by adaptive servo-controlled noninvasive ventilation, but clinical trials have failed to demonstrate any survival benefit and have even reported excess mortality [2]. Diaphragm pacing can stabilise breathing pattern in left-heart failure-related periodic breathing and the preliminary results of clinical trials suggest long-term benefits. However, diaphragm pacing is a highly specialized procedure that is not devoid of risks [3-5]. Pharmacological treatment of periodic breathing is therefore an attractive option. Oxygen, acetazolamide and theophylline have been investigated with contrasting results [6, 7], but substances known to interfere with the activity of respiratory central pattern generators have not been tested. Baclofen, a chemical derivative of γ-aminobutyric acid (GABA) that activates $GABA_B$ receptors in the central nervous system, may represent such a substance. It is widely used to treat severe spasticity in patients with spinal cord lesions [8] and, at higher doses, as an adjunctive approach to alcohol dependence [9-11].

GABAergic neurotransmission in general plays an important role in the neural mechanisms controlling the intensity of the neural drive to breathe [see review in12]. $GABA_A$ agonists generally have depressant respiratory effects [12]. $GABA_B$ activation (by baclofen) also has respiratory depressant effects in many mammalian species [13-15] —including in humans at high doses [16, 17]—, but excitatory effects have been described —in the rat— [18, 19]. GABAergic neurotransmission also plays a major role in the neuromodulation of respiratory pattern formation. This has been shown by numerous experiences involving superfusion or localized microinjections of $GABA_B$ agonists and antagonists in various *in vivo*, *in situ*, and *in vitro* models [20], with the general conclusion that $GABA_B$ signaling is instrumental in generating eupneic, continuous breathing rhythms [20]. In addition, several arguments suggest that GABAergic signaling plays an important role in the regulation of central apneas and of periodic breathing. Zolpidem, a $GABA_A$ receptor agonist of the imidazopyrine class, decreases the frequency of central apneas induced be altitude hypoxia in humans [21]. More specifically relevant to our study, $GABA_B$ receptors stimulation interferes with periodic breathing, as follows. Air breathing in frogs and their larva, the tadpole, is naturally periodic [22] and originates in brainstem neural oscillators [23], providing

a relevant model to study the pharmacology of periodic breathing. In a preparation of isolated tadpole brainstem [24], intermediate concentrations of baclofen transformed the periodic output of the cranial nerves controlling lung ventilation into a continuous output [25]. Finally, baclofen is effective at suppressing hiccoughs, either artificially induced in cats [26] or occurring spontaneously in humans [27], probably by restoring or reinforcing the inhibitory action of GABAergic inhibitory inputs originationg within the nucleus raphe magnus on the hiccup reflex arc [28]. All these effects suggest reconfiguration of respiratory central pattern generators, and are compatible with the notion that the pathophysiology of periodic breathing might involve central GABAergic dysregulation. In this regard, periodic breathing in left heart failure is generally interpreted as a consequence of brainstem hypoperfusion leading to hypoxia. Yet certain models show that hypoxia downregulates GABA in the central nervous system [29, 30]. Periodic breathing in left heart failure could thus proceed from central GABA deficit and/or relative$GABA_B$ insensitivity to endogenous GABA.

The present study therefore tested the hypothesis that baclofen could correct or attenuate the breathing instability resulting from hypoxia exposure during sleep in normal humans, namely in a situation known to be associated with the occurence of periodic breathing.

Methods

Study design and participants.

This study, code named *Periodibac*, was a single-centre, randomized, double-blind, cross-over controlled trial conducted in a 1600-bed university hospital in Paris, France. Participants were recruited consecutively by local advertising (Faculty of Medicine). Inclusion criteria were: age over 18; male gender; body mass index between 20 and 30 $kg.m^{-2}$; healthy subjects (no regular treatment apart from step 1 analgesics), who had not previously participated in any physiology experiments. Exclusion criteria were any self-reported current or past chronic or serious acute somatic disorder (respiratory or otherwise), self-reported current or past significant psychological or psychiatric disorder, smoking greater than 2 pack-years, use of any CNS active drug, alcohol or drug use, history of confirmed or possible acute mountain sickness, migraine, claustrophobia, known baclofen intolerance, and legal restriction to participate in biomedical research. The participants were instructed to refrain from taking any analgesic and anti-inflammatory medications, alcohol, caffeine, and any psychotropic substances for 48 hours prior to the experiments.

The study was conducted according to the principles of the declaration of Helsinki. It was approved by the appropriate ethical and regulatory bodies according to the French law (*Comité de Protection des Personnes Ile-de-France 8, Suresnes, France,* decision # 09 05 35 and *Agence Nationale de Sécurité du Médicament et des produits de Santé*) and was publicly registered (ClinicalTrials.gov NCT01095679). The study sponsor was the

Département de la Recherche Clinique et de l'Innovation (DRCI) of *Assistance Publique-Hôpitaux de Paris (AP-HP*). The participants received detailed information about the objectives of the study, the methods used, and the potential risks. They provided their written consent to participate and received a financial incentive for their participation.

Randomisation and masking

Participants eligible for the baclofen-placebo comparison (see below, protocol, and Figure 1) were randomized (RandoWeb online software, APHP, Paris, France - http://randoweb.aphp.fr/index.php) to receive either baclofen first and then an identical-looking placebo (specifically manufactured for the study) or placebo first and then baclofen (1:1 block randomization without stratification). Participants and all investigators were blinded to the treatment arm.

Procedures

Exposure to hypoxia

Participants were studied in a 2.3 m^3 hypoxia tent (portable queen size tent, Hypoxico altitude training system, New York, NY, USA) supplied by a hypoxia generator capable of generating 7.6 $m^3.h^{-1}$ (hypoxic generator, Everest summit II, Hypoxico altitude training System, New York, NY, USA). Inspired oxygen fraction (FiO_2) within the tent was regularly controlled (Handi+ oxygen analyser, Maxtec, Salt Lake city, Utah, USA) and maintained between 12 et 14% (corresponding to the inspired partial pressure of oxygen found at an altitude of 3,800 m). Note that tent ventilation did not allow complete CO_2 removal, resulting in a slightly hypercapnic atmosphere (inspired partial pressure of carbon dioxide 6.2 ± 1.4 mmHg [mean ±SD]).

Measurements

Ventilatory flow (V'_E) was measured by a differential pressure pneumotachograph (Hamilton Medical AG, PN 279331, Rhäzüns, Switzerland) connected in series with a face mask (total dead space of the apparatus: 170 mL). The partial pressure of carbon dioxide in the expired gas was continuously measured by an infrared gas analyser connected to a dedicated port of the face mask (Servomex 1505, Servomex, La Plaine Seine Saint-Denis, France). The corresponding end-tidal value ($P_{ET}CO_2$) was determined at the very end of the CO_2 expiratory plateau. Both signals were fed to an analogue-digital converter (MacLab 16S, PowerLab System, AD Instruments, Castle Hill, Australia; 200 Hz sampling rate) and recorded for subsequent analysis (Chart 7.1 AD Instruments, Castle Hill, Australia). The V'_E signal was used to determine tidal volume (V_T), breathing period (total cycle time, T_T), inspiratory time (T_I) and expiratory time (T_E).

Standard polysomnographic recordings were performed using a portable device (Dream, Medatec, Brussels, Belgium) collating three EEG channels, two electrooculogram channels (EOG), one electromyogram channel (chin), electrocardiogram, ventilatory flow from the mask-pneumotachograph device, thoracic and abdominal movements by means of

elastic belts, and pulsed oximetry. These recordings were used to identify and stratify sleep according to the usual classification (non-rapid eye movement —non-REM— sleep stages 1 to 3: N1, N2, N3 ; rapid eye movement sleep —REM—).

Protocol

All participants underwent a first hypoxia session ("hypoxia 1") to determine whether or not they developed perioding breathing in response to low inspired oxygen during sleep. Participants who did develop perioding breathing acording to visual inspection of the ventialtory tracings (clusters of breaths with or without a crescendo-decrescendo pattern separated by a period of central hypopnoea or apnoea or crescendo-descrescendo pattern only) without exhibiting signs of acute mountain sickness (Lake Louise score 0-2; [31, 32] were randomized to receive either the baclofen-placebo or the placebo-baclofen sequence. Treatment was administered orally at the final dose of 60 mg per day (20 mg x 3). In the absence of any indication in the literature regarding the dose to use in humans in order to modify breathing control, we used the daily dose that is recommended in France to treat spasticity in adults, and that therefore shoud be sufficient to exert an effect on the central nervous system. This dose was reached progessively (5mg x3 for 3 days, 10 mg x3 for 3 days, 15 mg x 3 for 3 days, and 20 mg x 3 for the last three days). The second hypoxia session was then performed ("hypoxia 2"), followed by a 5-10 day washout. The alternate treatment was then administered according to the same scheme, until the third and last hypoxia session ("hypoxia 3").

Hypoxia sessions were conducted in a quiet dark room during a nap following a light meal, starting consistently at 1:30 pm. To ensure that the participants would actually sleep during the recordings, they were asked to sleep deprive themselves for 24 hours before each session (this was checked by asking them to call the laboratory each hour and leave a message on an answering machine). During the hypoxia sessions, transcutaneous pulsed oxygen saturation, heart rate and blood pressure were monitored for safety purposes.

Outcomes.

The primary outcome of the study was the coefficient of variation (standard deviation divided by the mean) of the respiratory cycle period or total time ($CoVarT_T$) calculated during hypoxic sleep. $CoVarT_T$ describes the breath-by-breath variability of the temporal dimension of the respiratory central pattern generators output. An increase in $CoVarT_T$ denotes breathing irregularity and is a known response to hypoxia. It was expected to become significantly lower with baclofen than with placebo.

Planned secondary outcomes included the coefficient of variation of tidal volume ($CoVarV_T$) and of end-tidal partial pressure of carbon dioxide in the expired gas ($CoVarP_{ET}CO_2$), both describing the breath-by-breath variability of the intensity of the respiratory central pattern generators output. Other planned secondary outcomes were: *i*) the apnoea-hypopnoea index, defined as the number of interruptions and reductions of

ventilatory flow per sleep hour; *ii*) the sleep fragmentation index defined as the number of micro-awakening and awakenings per hour of sleep; *iii*) quantification of the nonlinear dynamics of the ventilatory flow signal by the noise titration procedure [33-35](briefly, this approach consists in fitting the analysed signal with an array of linear and nonlinear models ; if the signal is best fitted by a nonlinear model -complexity-, noise is added to the initial signal until the resulting one becomes best fitted by a linear model -titration-; the more noise required to obtain this result, the more complex the initial signal); and *iv*) quantification of frequency compounds of ventilatory flow assessed by a slow oscillation ratio derived from the Fast Fourier Transform (FFT) decomposition of the ventilatory flow signal (ratio between the mean amplitude of the 0.024-0.146 Hz band of the FFT to the mean amplitude of the peak frequency band of the FFT —namely resting breathing frequency—; an increased slow oscillation ratio indicates low frequency breathing instability, as expected during periodic breathing).

Statistical analysis

Sample size was calculated using simulations with SAS IML software, based on assumptions from the published literature that CoVarTT should be around 20 ± 10% in normoxia and 45 ± 30% in hypoxia [36, 37]. With these assumptions, 14 subjects exhibiting periodic breathing during hypoxic sleep would be necessary to demonstrate the ability of baclofen to restore the normoxic value of CoVarTT during hypoxia with 80% power and a two-sided type I error rate of 5%.

Data are presented as medians [interquartile range]. Data measured during the "hypoxia 2" and "hypoxia 3" periods (see above, protocol, and Figure 1) were all quantitative and compared with Wilcoxon signed-rank test after checking for the absence of carry-over (treatment/period interaction). A p value less than 0.05 was considered statistically significant. All comparisons were planned and were restricted to the population of subjects who actually participated in both "hypoxia 2" and "hypoxia 3".

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

Results

Participants

The characteristics of the 14 randomized participants are provided in Table 1. Figure 1 describes the study flow chart: only 11 patients participated in both the "hypoxia 2" and "hypoxia 3" sessions, and recording technical issues resulted in an excessive amount of missing data in 2 subjects. Statistical analysis was finally conducted on 9 subjects.

Primary outcome

Baclofen did not stabilise breathing during hypoxic sleep, as illustrated by Figure 2. On the contrary, CoVarTT significantly increased with baclofen compared to placebo, both during non-REM sleep considered as a whole (placebo: 56.00% [37.63 - 78.95]; baclofen: 85.42% [68.37 - 86.40], p=0.020) and during N1-N2 sleep (placebo: 59.91% [37.95 - 86.92]; baclofen: 98.09% [83.90-98.99], p=0.012), but not during N3 sleep (placebo: 34.07% [26.78 - 38.65]; baclofen: 36.36% [32.94-93.53], p=0.734) (Figure 3). On placebo, CoVarTT was higher during wakefulness than during sleep, with no significant difference between placebo and baclofen (77.59% [65.53 -82.51] and 76.34% [63.29 -89.29], p=0.910), respectively (Figure 3).

Other outcomes

CoVarVT increased significantly during N1-N2 sleep (placebo: 44.32 [32.47 - 50.00]; baclofen: 58.46 [42.73 - 67.12], p=0.027) (Figure 3), but not during wakefulness (placebo: 53.78 [49.33 - 65.35]; baclofen: 57.14 [44.04 - 60.26], p=0.652).

There was no significant difference in CoVar$P_{ET}CO_2$ during wakefulness or during sleep.

The apnoea-hypopnoea index and sleep fragmentation index were not significantly different between placebo and baclofen. The only indicator for which the difference was close to the limit of significance was the central apnoea index during non-REM sleep, which was 13.8/h [0.6 - 42.0] with placebo and 45.6/h [12.0 - 82.28] with baclofen (p=0.074).

During napping under hypoxic conditions, the noise titration procedure showed the non-linear and chaos-like dynamics of ventilation (positive noise limits). No significant difference was observed between placebo (noise limit: 37.80 [22.50-54.40]%) and baclofen (noise limit: 30.80 [14.50-51.90]%, p=0.605) and no significant difference was observed between placebo and baclofen in terms of the slow oscillation ratio (23.72 [15.16-32.10]% vs 20.58 [16.16-29.71]%, respectively; p=0.730).

Tolerability

None of the subjects reported any side effects related to administration of baclofen. Hypoxia was generally well tolerated: only 2 cases of a Lake Louise score of 2 were reported during all exposures to hypoxia, and none of the subjects reported a Lake Louise score of 3. Hypoxia-related symptoms rapidly resolved after the end of the procedure with no remanent effect.

Discussion

This study shows that, contrary to the hypothesis tested, but in line with anecdotal observations of baclofen-induced central sleep apnoea [16], baclofen did not stabilise breathing during hypoxic sleep. On the contrary, baclofen increased the variability of the ventilatory period TT during NREM sleep as a whole, an effect driven by the differences observed during N1-N2 sleep. Baclofen also increased the variability of tidal volume VT

during N1-N2 sleep. Together, these observations indicate that baclofen worsened breathing instability.

The effects of baclofen on the intensity of the mammalian neural drive to breathe vary between species. In cats, rabbits and guinea pigs, baclofen has inhibitory effects (decreased resting ventilation in rabbits [13] and guinea pigs [14]; depressed ventilatory response to carbon dioxide in cats [15]; depressed cough in cats [38, 39] and rabbits [40]). These effects are mediated via modulation of GABAergic inhibitory circuits involving the pre-Bötzinger complex [41, 42]. In rats, in contrast, the effects of baclofen on breathing control are excitatory (prolonged inspiratory time and increased diaphragmatic discharge [18]) and anti-inhibitory (depressed Hering-Breuer lung inflation reflex [19]). Of note, the ventilatory dose-response curve to baclofen can adopt an inverted U-shaped curve. In the cat, increasing the dose of baclofen from 0.5 to 4 mg/kg increased tidal volume between from 2 mg/kg to 4 mg/kg but decreased ventilation at 4 mg/kg [43], and while small doses of baclofen did not change or slightly increased the amplitude of phrenic nerve discharges, large doses profoundly decreased them [15]. In humans, baclofen overdose induces respiratory depression [17] and central sleep apnoea has been reported with repeated administration of high-dose baclofen in patients with alcohol use disorder [16] (and with standard-dose baclofen, but in a context of previous stroke, a condition known to predispose to central sleep apnoea [44, 45]). A single low dose of baclofen 25 mg) has been shown to have had no effect on the frequency of sleep-disordered breathing in patients with moderate obstructive sleep apnoea [46].

The effects of baclofen on the nature of breathing rhythmogenesis (as opposed to the intensity of the neural drive to breathe, see above) do not appear to have been studied in mammals. In amphibians, baclofen induces network reconfiguration, shifting the breathing pattern from periodic to continuous mode at intermediate concentrations [25]. However, at high concentrations, baclofen induces arrest of the ventilatory rhythm [25] (inverted U-shaped dose response pattern). In cats, baclofen suppresses hiccoughs induced by electrical stimulation in the medullary reticular formation [26]. Available clinical observations of the inhibitory effects of baclofen on hiccoughs in humans [27] combined with analogies between hiccoughs and gill breathing in tadpoles [47] can also be interpreted in terms of network reconfiguration. These data led us to think that baclofen could at least partially correct periodic breathing —or, in other words, stabilise breathing—, a hypothesis that was not supported by our findings. Our study does not provide any mechanistic explanation for the deleterious effects of baclofen on breathing stability, but it was not designed to do so. Animal studies would be necessary to achieve this goal. We acknowledge that it was probably excessively audacious to base our "human hypothesis" on data obtained in a model of isolated *Rana catesbaiana* brainstem. However, it must be kept in mind that the effect of baclofen on respiratory rhythmogenesis in the frog is highly dose-dependent: we cannot rule

out that the dosage used in our study was inadequate, and that other dosages may have had different effects (see below, limitations).

In contrast with the results reported by Gagnadoux *et al*. with high-dose oral baclofen [16] or Bensmal *et al*. with intrathecal baclofen [48], we did not observe prolonged central sleep apnoea (even though the central apnoea-hypopnoea index in our subjects was higher with baclofen, but not significantly so). This finding may simply be due to the use of standard-dose baclofen, and the nature of the study population (our subjects did not take any drug known to promote central sleep apnoea —e.g. opioids— [49] and did not present any of the conditions commonly associated with central sleep apnoea, e.g. heart failure, stroke, etc.). As hypocapnia and alkalosis can promote sleep-related instabilities [50], it could be speculated that accentuation of the ventilatory instabilities observed in this study may have been due to the combined effect of baclofen and hypocapnia, rather than to the action of baclofen *per se*. In this regard, we failed to achieve sufficient tent ventilation to allow optimal CO_2 clearance (see Methods, under "Procedures"). Consequently, hypoxic stimulation was conducted under slightly hypercapnic conditions, which makes it unlikely hypocapnia-induced instability interfered with our observations, which we therefore attribute exclusively to the pharmacological action of baclofen.

The major methodological strength of our study lies in its design, combining an innovative physiological approach to induce breathing instability and a randomized, controlled, double-blind design. The choice of CoVarTT as primary outcome could be criticized insofar as it is not a validated descriptor of periodic breathing, but it is a validated descriptor of breathing instability, and data are available for sample size calculation. Attrition due to missing data constitutes a weakness of the study. We had calculated that 14 subjects exhibiting periodic breathing during hypoxic sleep would be needed to achieve our power objective, and we stopped recruitment once this number was reached. Unfortunately, the final dataset pertained to only 9 subjects due to various factors (Figure 1). The mere existence of a statistically significant difference despite this data attrition attests to the strength of the effect. Furthermore, baclofen had consistent effects on both CoVarTT and CoVarVT: this reassuringly suggests that baclofen did actually interfere with the output of the central pattern generators.

The major limitation of our study is that there is no way to know whether the dose that we selected based on the treatment of patients with spatiscity is associated with baclofen concentrations in the central nervous system relevant to have an impact on period breathing in healthy subjects. The effects that we observed were probably not due to the use of too low a dose, given the description of central apneas at higher doses [16]. This dose used could however have been too high, either because of an inverted U-shaped dose response profile (see above) and/or due to a left shift in the dose response in healthy volunteers compared to patients with spasticity who often have multiple comorbidities and concomitant medications.

In the absence of previous studies of the effects of baclofen on periodic breathing in mammals, it was not possible to define a target plasma concentration in healthy humans. In addition, baclofen pharmacokinetics exhibits a very high interindividual variability in normal subjects [51], patients with alcohol use disorders [51], and patients with spasticity [52]. Nevertheless, we acknowledge that checking the effecs of low dose baclofen on hypoxia-induced periodic breathing by conducting a dose rising study would be necessary to draw a definitive conclusion.

Whatever the mechanisms by which baclofen increases breathing variability during sleep under hypoxic conditions, and although we used an experimental model in healthy subjects that prevents direct extrapolation to patients, the results of our study constitute a warning concerning the use of standard-dose or high-dose baclofen in patients with periodic breathing or at risk of developing periodic breathing. This includes cardiac failure patients, noting that in their case impaired renal function by low cardiac output would carry the risk of overdosage insofar as baclofen is predominantly excreted unchanged by the kidney. More generally, it would also appear reasonable to evaluate the risk of sleep-disordered breathing in patients considered for baclofen treatment, e.g. for spasticity on in the context of alcohol withdrawal, and to pay attention to the possibility of sleep-disordered breathing in patients actually receiving this treatment.

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Authors' contributions

Conception and design of the work:
CS, MT, SM, LB, BChe, TS
Acquisition and analysis of date:
MT, SM, NW, DH, CG, BCha, ED, NS, VA
interpretation of data:
CS, MT, SM, BChe, VA, TS
Drafting the work or revising it critically for important intellectual content:
CS, MT, DH, BC, LB, B Cha, BChe

Final approval of the version to be published:
All authors
Agreement to be accountable for all aspects of the work:
All authors

Conflict of interest statement

The study did not involve any conflict of interest, financial or otherwise.
Outside the study, Dr. ATTALI reports personal fees from Nyxoah, personal fees from Resmed, personal fees from ADEP; Dr. SIMILOWSKI reports personal fees from AstraZeneca France, personal fees from Boerhinger Ingelheim France, personal fees from GSK France, personal fees and non-financial support from Novartis France, personal fees from TEVA France, personal fees from Chiesi France, personal fees from Lungpacer Inc, personal fees from ADEP Assistance, grants from Air Liquide Medical Systems. The other authors report no conflict of interest.

Data availability statement

The data will be made available to external researchers by the authors upon reasonable request.

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Table 1. Characteristics of the 14 randomized participants on inclusion

Variables	n	median	[Q1; Q3]
General characteristics			
age (years)	14	24	[22.25; 28.75]
height (cm)	14	180.50	[178.25; 182.00]
weight (kg)	14	74	[68.25; 78.53]
body mass index ($kg.m^{-2}$)	14	22.62	[20.74; 25.05]
diastolic arterial pressure (mmHg)	14	63.50	[61.25; 72.25]
systolic arterial pressure (mmHg)	14	116.00	[108.00; 124.00]
Pulmonary function tests			
total lung capacity (% predicted)	14	104.00	[96.25; 111.75]
functional residual capacity (% predicted)	14	108.00	[96.25; 121.00]
vital capacity, VC (% predicted)	14	114.00	[100.75; 120.00]
forced expiratory volume in 1s, FEV_1 (% predicted)	14	108.50	[98.00; 114.50]
FEV_1/VC (% predicted)	14	97.50	[92.25; 100.75]
residual volume (% predicted)	14	91.00	[79.25; 108.50]
Other			
normal electrocardiogram	14	100%	
normal chest X-ray	14	100%	
transcutaneous pulsed oxygen saturation ≥ 96%	14	100%	

Figure legends

Figure 1. Study flow chart.
"*Other treatment, n=1*" means that the subject took another drug than baclofen during the study, which was prohibited by the protocol.
"*Recording issues, n=2*" refers to technical issues making the physiological recordings impossible to use.
"*Lost study treatment, n=1*" means that the subject lost the tablets he had been given and thus could therefore not continue to participate in the study.

Figure 2. Representative example, in one participant, of a bout of ventilatory recording obtained during N3 sleep under hypoxia with placebo (top) and baclofen (bottom). The recording obtained with baclofen shows one episode of periodic breathing vs. none on the recording obtained with placebo, and demonstrates an increased breath-by-breath variability.

Figure 3. Coefficient of variation of the total cycle time (CoVarT$_T$, top row) and tidal volume (CoVarV$_T$, bottom row) with placebo (red boxes) and baclofen (green boxes) during non-REM sleep as a whole, N1-N2 sleep, and N3 sleep. The boxes represent the interquartile range with indication of the median, the whiskers delineate the 95th percentile of the distribution range.

Figure 1

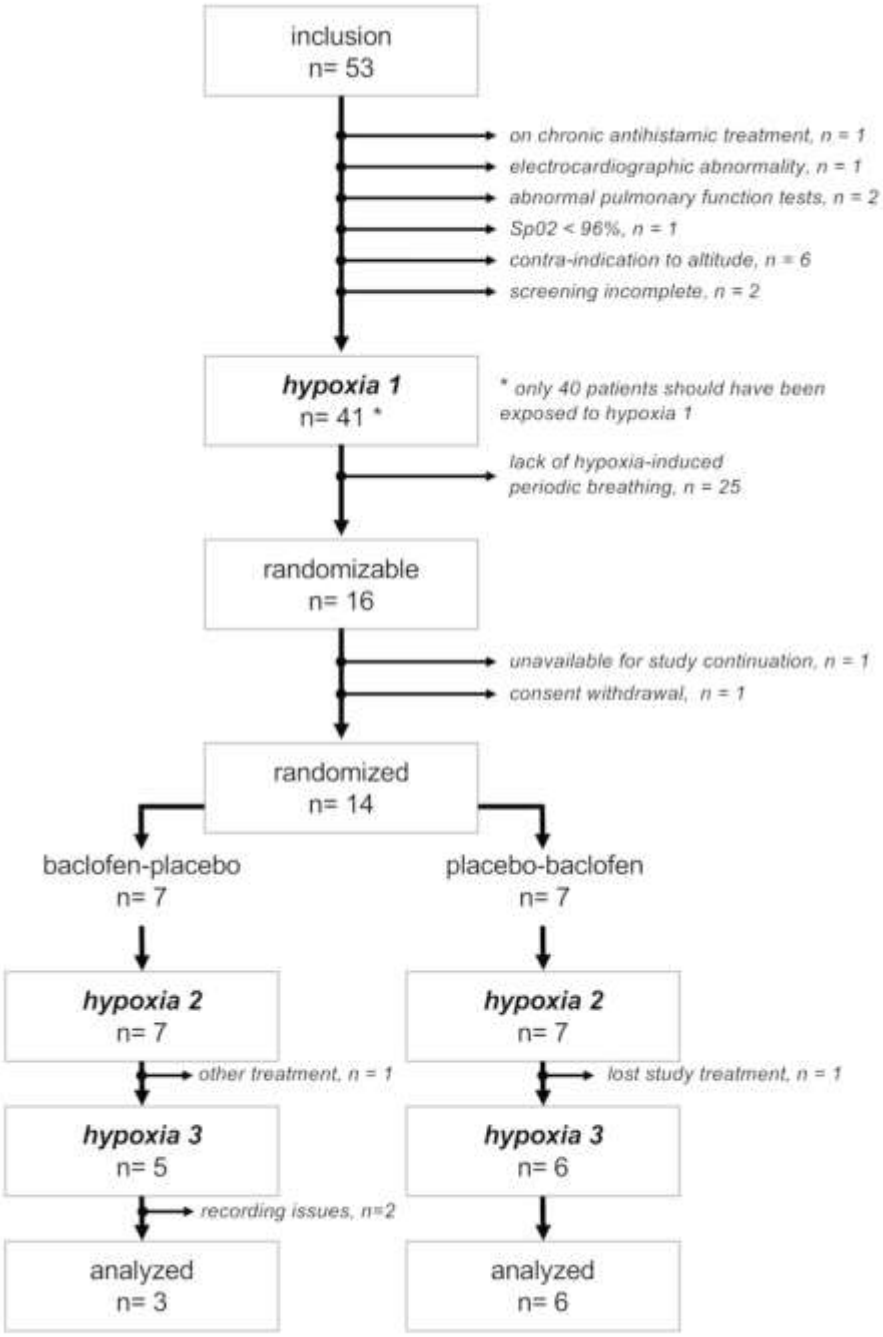
inclusion
n= 53
on chronic antihistamic treatment, n = 1
electrocardiographic abnormality, n = 1
abnormal pulmonary function tests, n = 2
Sp02 < 96%, n = 1
contra-indication to altitude, n = 6
screening incomplete, n = 2
hypoxia 1
n= 41 *
* only 40 patients should have been exposed to hypoxia 1
lack of hypoxia-induced periodic breathing, n = 25
randomizable
n= 16
unavailable for study continuation, n = 1
consent withdrawal, n = 1
randomized
n= 14
baclofen-placebo
n= 7
placebo-baclofen
n= 7
hypoxia 2
n= 7
hypoxia 2
n= 7
other treatment, n = 1
lost study treatment, n = 1
hypoxia 3
n= 5
hypoxia 3
n= 6
recording issues, n=2
analyzed
n= 3
analyzed
n= 6

Figure 2

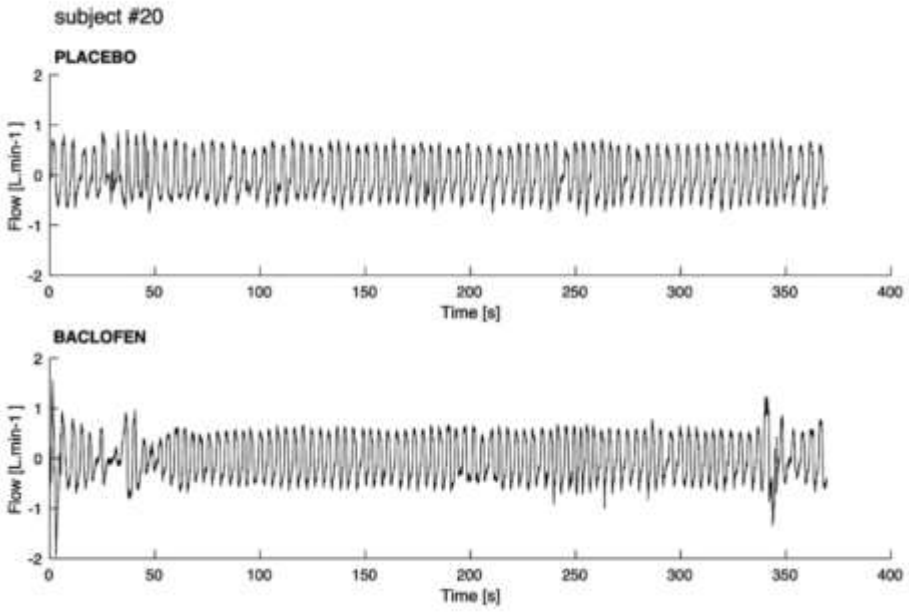

Figure 3