THE USE OF WHOLE BODY PLETHYSMOGRAPHY TO EVALUATE DRUG-INDUCED CHANGES IN RESPIRATORY DISORDERS: SLEEP APNEAS, COUGH, BRONCHOSPASM

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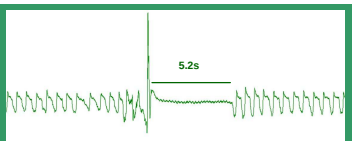
INTRODUCTION

Drugs from a variety of pharmacological classes are known to adversely affect the respiratory system. Whole body plethysmography (WBP) is widely used in safety pharmacology to investigate in conscious animals the potential undesirable pharmacodynamic effects on respiratory function. The aim of this study is to show that WBP could also be used to study drug-induced changes in respiratory disorders that could be life-threatening or associated with significant morbidity, such as sleep apneas, cough or bronchospasm.

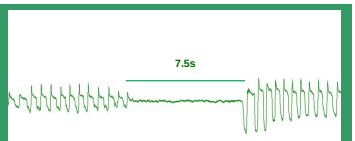
SLEEP APNEAS

Animals

- Male Sprague Dawley Rat / 250-300g
- Standard housing conditions:
  - Room temperature :22 ± 2°C
  - Hygrometry: 55 ± 10%
  - Light/dark cycle: 12h/12h
  - Tap water and food (SAFE A04): *ad libitum*



Post sigh apnea



Spontaneous apnea

Experiments

• The day before experiment, rats were placed in the plethysmograph chamber for at least a 1-h period for habituation to the experimental conditions

• The day of experiment, after a stabilisation period of at least 30 min, diazepam (5mg/kg) or its vehicle were administered orally (10 mL/kg)

• Sleep apneas were recorded for 5 hours, from 30 minutes post administration (required time for rats to fall asleep). Apneas parameters (total number, total duration and frequency) were therefore determined between 0H30 and 5H30 (0H30-1H30; 1H30-2H30; 2H30-3H30; 3H30-4H30; 4H30-5H30)

• Only sleep apneas with a duration of more than 2.5 s were retained for analysis and classified as follows:

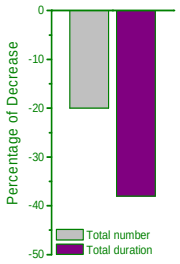
- 2.5s ≤ duration < 3.5s
- 3.5s ≤ duration < 4.5s
- 4.5s ≤ duration

Number of apneas over the 5-hour period for each class (n=18)

Apnea Duration (s)	0H30 - 1H30	1H30 - 2H30	2H30 - 3H30	3H30 - 4H30	4H30 - 5H30	*Total*
[2.5 - 3.5 [	3.4 ± 0.5	4.3 ± 0.4	3.8 ± 0.6	5.2 ± 0.7	4.6 ± 0.9	***21 ± 2***
[3.5 - 4.5 [	1.0 ± 0.3	1.8 ± 0.5	1.6 ± 0.4	2.0 ± 0.4	3.3 ± 0.6	***10 ± 1***
>= 4.5	0.3 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.7 ± 0.4	1.3 ± 0.5	***3 ± 1***
Total	***5 ± 1***	***7 ± 1***	***6 ± 1***	***8 ± 1***	***9 ± 1***	***34 ± 3***

Total duration of apneas over the 5-hour period (s): **117 ± 12**

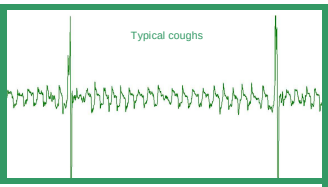
Effect of diazepam on apneas
(5mg/kg, p.o., n=5)



COUGH

Animals

- Male Dunkin Hartley Guinea Pigs / 250-500g
- Standard housing conditions:
  - Room temperature :22 ± 2°C
  - Hygrometry: 55 ± 10%
  - Light/dark cycle: 12h/12h
  - Tap water and food (SAFE 106): *ad libitum*



Experiments

• The day of experiment, animals were placed in a plethysmograph chamber

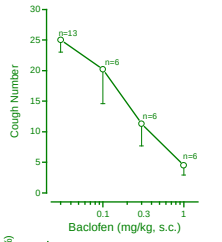
• Cough was induced by inhalation of an aerosol of 0.4 M citric acid solution

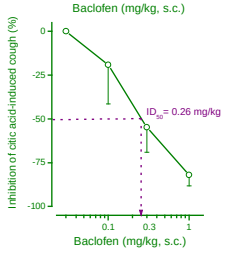
• Citric acid was administered for 12 min with an ultrasonic nebulizer connected to the air-flow port of whole body plethysmography boxes

• The number of coughs over the last 10 min of nebulization was counted by a trained experimenter, based on characteristic animal posture, sound produced and respiratory signal

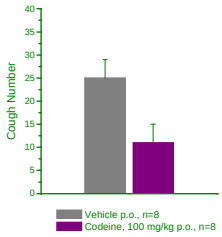
• Baclofen (0.1-0.3-1 mg/kg, s.c.), codeine (100mg/kg, *p.o.*) or vehicles were administered 30 min before starting citric acid nebulization

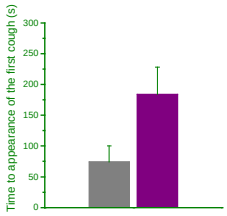
Effect of baclofen on citric acid-induced coughs





Effect of codeine on citric acid-induced coughs

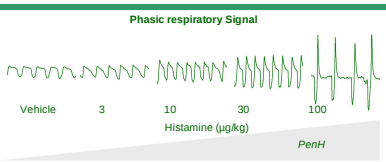




BRONCHOSPASM

Animals

- Male Dunkin Hartley Guinea Pigs / 250-500g
- Standard housing conditions:
  - Room temperature :22 ± 2°C
  - Hygrometry: 55 ± 10%
  - Light/dark cycle: 12h/12h
  - Tap water and food (SAFE 106): *ad libitum*



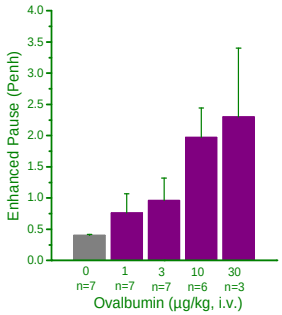
Experiments

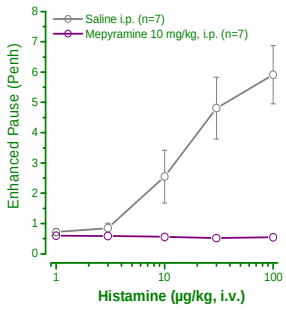
• **Ovalbumin-Sensitised animals:** guinea pigs were administered 3 weeks before experiment with 0.5 ml of saline containing ovalbumin (20 µg/ml s.c.) and 0.5 mL of aluminium hydroxide (200 mg/ml i.p.)

• **Surgical procedure for intravenous admnistration:** the day before the experiment, animals were anaesthetised with isoflurane (2.5-5%) and a heparinised saline-filled catheter was introduced into the left jugular vein. Catheter was tunneled subcutaneously and externalised at the interscapular area. Experiments were performed in conscious animals the day after

• **Experimental protocol:** all animals were pre-treated with propranolol (1mg/kg i.v.) 30 min before the challenge.
  - In sensitised animals, successive increasing doses of ovalbumin (1-3-10-30 µg/kg i.v., 1mL/kg) were injected at 10-minute intervals
  - In non-sensitised animals, successive increasing doses of histamine (1-3-10-30-100 µg/kg i.v., 1ml/kg) were injected at 10-minute intervals. Mepyramine (10mg/kg, 1ml/kg i.p.) was administered 30 min before the challenge with histamine

• **Analysis:** enhanced pause (Penh) was calculated by means of HEM software (Notocord) according to the following formula: Penh = (Te-RT/RT) x (PEF/PIF), where RT = relaxation time evaluated as the time to exhale 64 % of the tidal volume.





CONCLUSION

These results show that whole body plethysmography is appropriate to evaluate the effects of new chemical entities on respiratory disorders such as sleep apneas, cough and bronchospasm.

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