INVOLVEMENT OF GABAERGIC NEUROTRANSMISSION IN NEUROCHEMICAL DISTURBANCES UNDER A SINGLE OR REPETITIVE NITROGEN NARCOSIS

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Nitrogen narcosis by Junod, 1835:

« the functions of brain are activated, imagination is lively, thoughts have a peculiar charm and, in some persons, symptoms of intoxication are present »

INTRODUCTION

Nitrogen Narcosis

Exposures to compressed air induced a neurological syndrome composed by motor and cognitive disturbances correlated to the increase partial pressure of nitrogen (Behnke et al, 1935).

Neurochemical studies in rats demonstrated a decrease of the dopamine (**DA**) release in the dorsal striatum, a subcortical structure involved in the regulation of motor and locomotor processes which are disturbed by nitrogen narcosis (Bennett and Rostain, 2003).

Repetitive exposures to nitrogen narcosis

Interestingly, repetitive exposures to nitrogen narcosis produced a reversal and increasing effect on DA release (+10%), without diminished motor disturbances (Lavoute et al, 2005). Moreover, behavioural studies in men indicated only a subjective adaptation (Hamilton et al, 1995)

Neurochemical studies in rats

FIRST EXPOSURE TO NITROGEN (3MPa): (-20%)

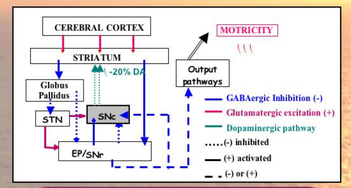
Comparisons with others gases

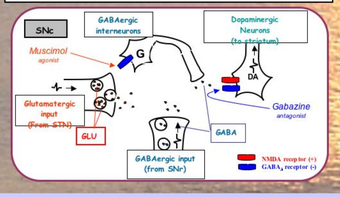
Other gases	CHARACTERISTICS	PRESSURE	DA LEVEL
NITROGEN	Anesthetic under pressure	(3MPa)	\ (-20%)
Nitrous Oxide	Anesthetic Gas	(0.1 MPa)	\ (-20%)
Argon	Anesthetic under pressure	(2MPa)	\ (-20%)
Helium	Non Anesthetic	(3MPa)	/ (+20%)

®The decrease dopamine under nitrogen pressure is attributed to a narcotic effect, rather than pressure effect

SECOND EXPOSURE TO NITROGEN (3MPa)
AFTER 5 REPETITIVE EXPOSURES TO 1MPA
// DA RELEASE (+10%)

Basal ganglia network

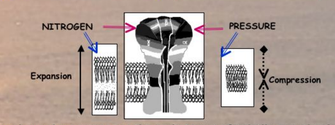




Action mechanisms of anesthetic gases

1-Lipid theory:
Molecules of gas dissolved in lipidic bi-layers of membrane cells (Miller *et al.* 1973)

2-Protein theory:
binding/interaction with ion-channel receptors (Franks and Lieb, 1994)



Ion-channel receptors are the main target of volatile anesthesics

NITROUS OXIDE ⇒ **antagonist of NMDA receptors** (Jevtovic-Todorovic, 1998; Balon *et al*, 2003)

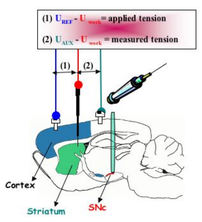
NITROGEN ⇒ **is not an antagonist of NMDA receptors** (Lavoute *et al*, 2006)

What about **GABA$_A$ receptors** ???

MATERIALS AND METHODS

Animal preparation and Surgery

Under general anesthesia, Sprague-Dawley male rats were stereotaxically implanted with dopamine-sensitive electrodes (**working electrode**) in the dorsal striatum, and bilaterally with cannulae in the substantia pars compacta (SNc) for drug injections. **Reference** and **auxiliary** electrodes were fixed on the bone.

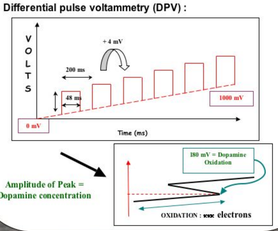


Measurements of dopamine level

Electrochemical signals were applied through a three electrodes potentiatic system **(1)** and received signal from oxidation **(2).**

- ✓**Every 3 minutes**
- ✓**In freely-moving rats**
- ✓**In normobaric / hyperbaric conditions**

➢ **Differential pulse voltammetry (DPV) :**



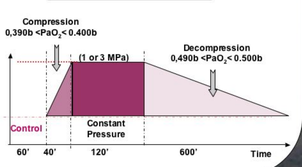
Amplitude of Peak = Dopamine concentration

Protocol of nitrogen exposure

After recovery from surgery (1 week) and stabilization electrode's basal response

FIRST EXPOSURE	REPETITIVE DAILY EXPOSURES TO NITROGEN					SECOND EXPOSURE
Nitrogen (3 MPa)	D_1 1MPa	D_2 1MPa	D_3 1MPa	D_4 1MPa	D_5 1MPa	Nitrogen (3 MPa)

Pressure exposure



Nitrogen pressure equivalences

ANESTHETIC THRESHOLD (NITROGEN)

RATS		MEN	SYMPTOMS
1MPa	25%	0.3MPa	Appearance threshold
3MPa	75%	0.8MPa	Well established
4.15MPa	100%	1.2MPa	-Loss of consciousness -Loss righting reflex

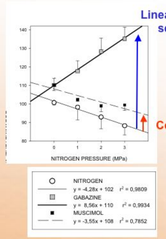
Drug injections

Drugs were dissolved in PBS and injected bilaterally in SNc 30 minutes prior to compression at a rate of 0.1µl/min for 5 minutes.

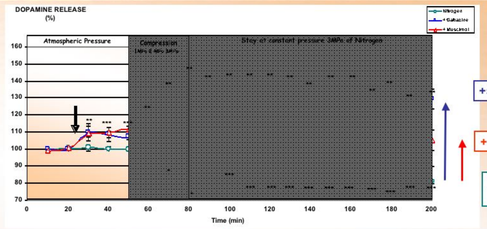
- ✓10^{-8} mol of Muscimol (specific **agonist GABA$_A$** receptor)
- ✓10^{-6} mol of Gabazine (specific **antagonist GABA$_A$** receptor)

RESULTS

1st EXPOSURE TO NITROGEN NARCOSIS (3MPa)



® Under compression phase,
linear increase of GABAergic input through GABA$_A$ receptors to dopaminergic neurons.



Effect of GABA$_A$ agonist injection at 3MPa nitrogen

Muscimol counteracted the **nitrogen**-induced decrease of dopamine, which returned to control values.

Functional GABA$_A$ receptors located **on GABAergic interneurons** with **unchanged sensitivity.**

Effect of GABA$_A$ antagonist injection at 3MPa nitrogen

Gabazine reversed the nitrogen-induced decrease of dopamine and increase it **(+35%),** during the whole exposure to nitrogen.

® **Functional** GABA$_A$ receptors located **on dopaminergic neurons** with **increased sensitivity**

AFTER 5 REPETITIVE EXPOSURES TO 1MPA :

2nd EXPOSURE TO NITROGEN NARCOSIS (3MPa)

Effect of GABA$_A$ agonist injection at 3MPa nitrogen

Muscimol **enhanced** the increasing effect of repetitive exposures to nitrogen

®**functional GABA$_A$** receptors located **on GABAergic interneurons**

® **Hypothesis 1: increased sensitivity of GABA$_A$ receptors located on GABAergic interneurons**

® **Hypothesis 2: desensitization of GABA$_A$ receptors located on DA cells**

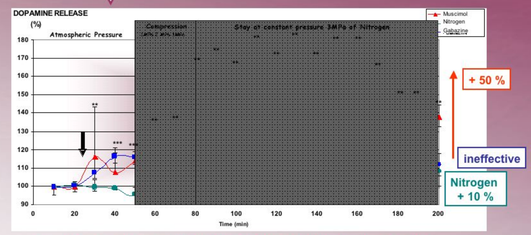
Effect of GABA$_A$ antagonist injection at 3MPa nitrogen

Gabazine remained **ineffective** to modify the increasing effect of repetitive exposures to nitrogen.

®**Dysfunction** and/or **desensitization** of **GABA$_A$ receptors located on dopaminergic** neurons under nitrogen exposure.

and/or

® **Loss of GABAergic input to DA cells**



Consequently, repetitive exposures to nitrogen induced a desensitization of GABA$_A$ receptors located on DA cells (hypothesis 2)

DISCUSSION

Nitrogen action under a single exposure

The **nitrogen-induced decrease** of the striatal dopamine release **(-20%)** is due to a facilitation of the GABAergic input to dopaminergic neurons, mediated by GABA$_A$ receptors in SNc.

A hypersensitization of GABA$_A$ receptors by nitrogen is suggested, as compared to:

-**alcohols** (Davies and Alkana, 2001)

-**Volatile anesthetics** (Belleli et al, 1999)

-**Solvents such as toluene** (Mihic et al, 1997)

AFTER REPETITIVE EXPOSURES, Nitrogen action under a second exposure

The repetitive nitrogen exposure-induced increase of the striatal dopamine release **(+15%)** is due to a decrease of the GABAergic neurotransmission mediated by GABA$_A$ receptor located on dopaminergic neurons in SNc. (desinhibitory process)

A desensitization of GABA$_A$ receptors to nitrogen is suggested.

This action could be comparable to repetitive exposures to:

-**alcohols** (Cagetti et al, 2003)

-**Solvents** such as toluene (Williams et al, 2005)

CONCLUSION

1) **Nitrogen could act directly on GABA$_A$ receptor by potentiating its activity, such as a co-agonist, underlying the binding-protein theory of inert gases.**

2) **Repetitive exposures to nitrogen may induced a nitrogen addiction, such as drugs, which may underlie a decreased responsiveness of GABA$_A$ receptor.**

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