J. Expt. & Clin Anat. 1(1): 16 – 18 (2002).

JECA

Response Of Decerebrate Piglets Dialysed With Muscimol To Hypercapnic Challenge.

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ABSTRACT

The pathogenesis of sudden infant death syndrome (SIDS) is hitherto poorly understood. It is, however, often associated with developmental abnormalities in the brainstem of victims. This study attempted to define the extent of compromise in central chemo sensation and response to hypercapnic challenge in piglets using an inhibitory neurotransmitter, GABA that is commonly found in the brainstem.

The results showed a reduction in the ventilatory output indices of the animals. The results suggest a further mechanism by which central abnormalities might underlie reduced respiratory control as occurs in sudden infant syndrome.

Key Words: sudden infant death syndrome, piglets.

The pathophysiology of sudden infant death syndrome (SIDS) is largely unknown. Many studies have demostrated developmental abnormalities in the brainstem of SIDS victims. These abnormalities include:

(a) Developmental hypoplasia of the arcuate nucleus (AN) (Filiano & Kinney, 1990).

(b) Reduced muscarinic receptor binding (Kinney et al, 1995),

(c) Reduced kainite receptor binding all in the arcuate nucleus (Filano et at, 1990).

The arcuate nucleus is a thin layer of cells on the ventral surface of the human medulla, most extensive rostrally. The AN has homologues in the chemosensitive area of the ventral medulla of animals (Nattie & Li, 1996).

These areas are collectively referred to as rostral ventral medulla (RVM), which controls breathing in piglets.

This study aims to define the effect of application of the $GABA_A$ receptor agonist – muscimol via microdialysis, on ventilatory output, and the response to systemic CO_2 challenge in decerebrate piglets. $GABA_A$ is a post – synaptic inhibitory neurotransmitter that is ubiquitous in the central nervous system.

The use of muscimol was to inhibit cells within the RVM mimicking arcuate nucleus hypoplasia previously described for a subset of SIDS victims. We hypothesized that such an intervention would profoundly inhibit baseline phrenic nerve (PNA) and reduce responses to systemic CO_2 challenge. Such a result would suggest a mechanism by which hypoplasia and reduced receptor binding in the AN might contribute to the pathogenesis of SIDS. And the result may offer further insights into the pathogenesis of sudden infant death syndrome.

METHODS AND MATERIALS

15 – 20 day old piglets from our onsite animal house were used. Animals were anaesthetized with 2% halothane (NJ, USA) in O_2. Body temperature was maintained at 38 – 39^{0}C using a heating pad and monitored by a rectal probe; femoral arterial and venous catheters were inserted for measurement of arterial blood pressure and the administration of drugs respectively. Each animal was tracheostomised and artificially ventilated (Harvard App. Dual Phase Respirator, MA, USA) to maintain end tidal carbondioxide ($ETCO_2$) at 5%. The animals were positioned in a sterotaxic apparatus for deceberation. Following decebration, halothane was discontinued.

Supplemental dose of pancuronium bromide 0.5 mg/kg/hr., administered when required, was used to paralyze the animals. The phrenic nerve was exposed and placed

on bipolar recording electrodes to monitor respiratory output. Phrenic Activity was amplified and moving time averaged 100ms time constant (CWE, Ardmore, USA). Peak integrated Nerve Activity (PNA), Phrenic Frequency (FR), and Phrenic Minute Activity (MA) were captured on a computer based Data Acquisition software/system – power lab (ADI Australia) and later analyzed.

Muscimol Dialysis

A microdialysis guide tube was guided into the RVM. A microdialysis probe was then introduced to about 1 – 1.5mm from the ventral surface of the brainstem. The RVM was dialyzed at the rate of 8.5 microlitres per minute within an artificial cerebrospinal fluid (aCSF) which was equilibrated with 5% Co_2.

A 60 minutes period was allowed to elapse before the hypercapnic challenge whereas a 30 minutes period was allowed for recovery. The aCSF in the dialysis system was replaced with 10millimolar muscimol 30 minutes before repeating the CO_2 challenge of 8%.

RESULTS

The correct localization of the probe was established by a post – muscimol dialysis of $KMN0_4$ stain and is confirmed by existing computer stereo localization data (Fig 1). Our results showed that in decereberate piglets, muscimol dialysis of the RVM substantially reduced the:

(a) PNA and the

(b) MA, and caused a fall in the

(c) FR (breaths / minutes)

These findings are shown in Figs 2 and 3. The figures show the plots of PNA, MA and fR before and after an 8% CO_2 challenge.

There were in particular, downward displacements of the responses following challenge with $ETCO_2$ at 8% but without much effect on its slope. The depression of the rate of breaths/min (fR) in particular, suggests that the inhibition of the RVM by muscimol depresses/distorts the control of breathing. Breathing rate and phrenic activities normally rose with increases in the $ETCo_2$ of decerebrate piglets dialyzed with artificial cerebrospinal fluid (aCSF) as had been previously shown (Kinney et at, 1995).

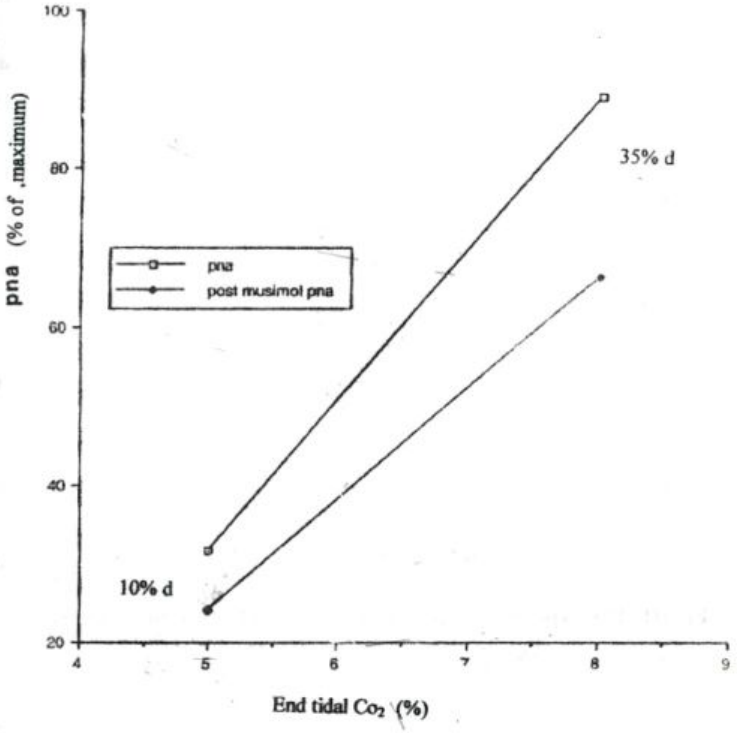

Fig I: Integrated phrenic nerve activity in decerebrate piglets challenged with hypercapnia

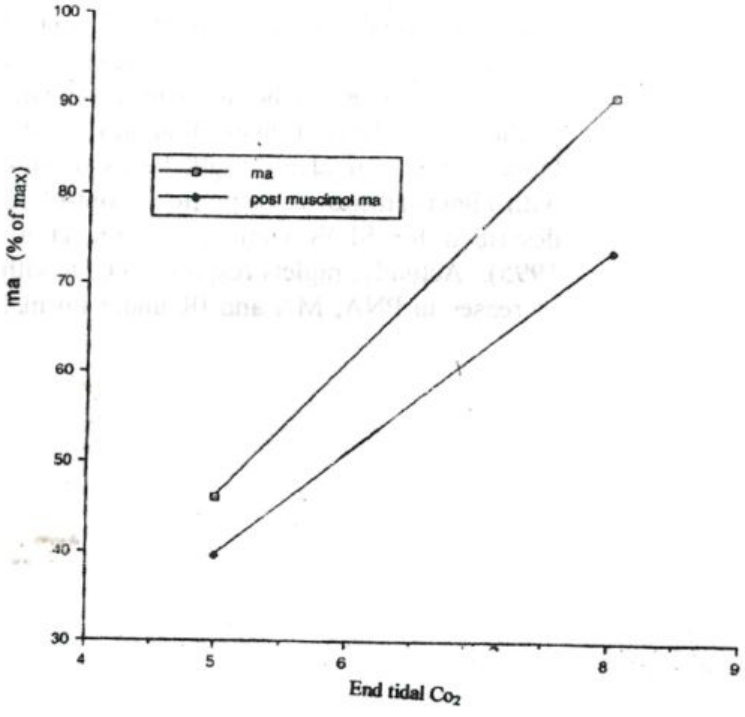

Fig II: Phrenic minute activity (ma) in decerebrate piglets challenged with hypercapnia

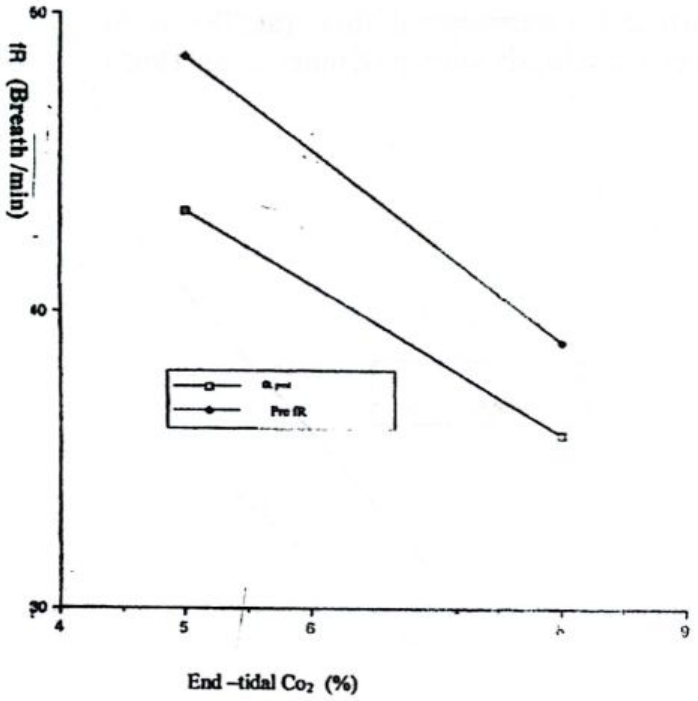

Fig III: The hypercapnic phrenic frequency of decerebrate piglets

DISCUSSION

. The RVM is involved in the neural control of breathing in piglets. This has been fairly well established (Nattie, 2000). The hypoplasia/hypo function of this area can cause abnormalities in respiratory control and responses to CO_2 challenges in particular. The RVM homologue in humans is the AN. Thus a hypo function of the human arcuate nucleus might be associated with abnormalities in respiratory control as described for SIDS victims (Kinney et al, 1995). Actually, piglets respond to CO_2 with increases in PNA, MA and fR under normal conditions. But when their control centres i.e RVM have been inhibited, as in this present study, by muscimol these responses to $ETCO_2$ of 8% captured as phrenic nerve activity and its minute activity and breaths per minute were all depressed. Dialysis of aCSF in the control group produced no changes in slope and values (fig II and III). Our findings corroborate the rather built – up fact of a central chemoreception centre but it shows that physical and physiological mal functioning of such centres impair respiratory responses in times of challenges. These findings thus, in general, situate at least a subset of sudden infant death syndrome possible pathogenic basis to impairments in central centres for respiratory control. It is reportedly possible that multiple sites mediate chemoreception and control in man (Nattie, 2000) and piglets.

ACKNOWLEDGEMENTS:

Many thanks to the SIDS LAB, DHMC for the opportunity and access.

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