Gestational hypoxia alone or combined with restraint sensitize the HPA axis and induce anxiety-like behavior in adult male rat offspring

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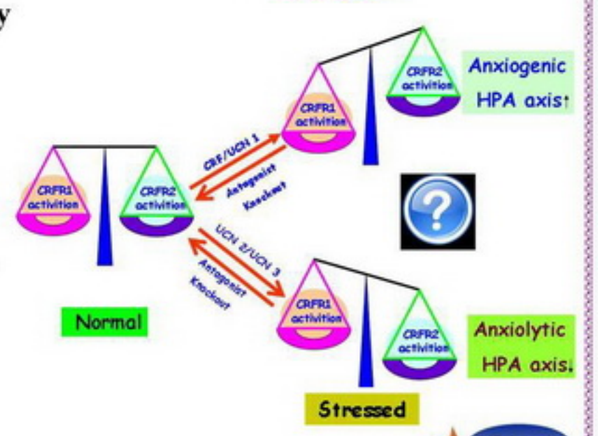
Introduction

High altitude hypoxia has long been considered important because large populations of people live at high altitude, and many others like to visit for trekking and climbing or athletic training; and the hypoxia due to sleep apnea is also of concern. Hypoxic stress may be acute, chronic or intermittent. Clinical studies have demonstrated that hypoxia/ischemia during pregnancy occurs in many pathological conditions, including maternal anemia, hypertension disorder complicating pregnancy, obstructive sleep apnea syndromes, umbilical cord occlusion, reduced placental size and decreased uteroplacental blood flow. Hypoxia also occurs in some physiological conditions in pregnant women, including living, visiting or training at high-altitude hypoxia, maternal smoking and alcohol consumption. Maternal hypoxia in pregnancy has been reported to be one of the most important putative noxious signals occurring during development, which has long lasting consequences for the fetus, infant and adult. In addition, exposure of pregnant animals to different social environments during pregnancy, as with the validated model of prenatal restraint (R) stress, has permanent behavioral and neurobiological consequences. Therefore, to explore how more natural environmental stresses in humans during pregnancy influence offspring, we set up natural models mimicking physiological stress (hypoxia) and psychological stress (R) or a combination of both, to investigate how the maternal stress of hypoxia and/or R impact on the function of the HPA axis and behavior of offspring.

CRH is the critical regulator of the HPA axis, and the endocrine, autonomic, and behavioral responses to stress. CRH dysfunction is implicated in anxiety and depression. CRHR1 has a high affinity for CRH or urocortin I (Ucn I), and CRHR2 has a high affinity for Ucn II and Ucn III. CRHR1 is believed to be crucial in stress-induced HPA responsiveness and anxiety-like effects. In contrast, CRHR2 seems to mediate anxiolytic-like effects.

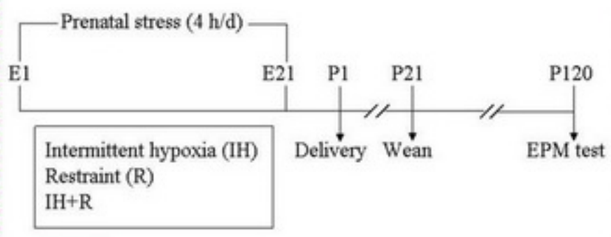
Hypothesis

In the present study, we wished to clarify our hypothesis that maternal pregnant intermittent hypoxia alone or in combination with restraint may lead to changing the HPA activity and modify the behavior in adulthood of their offspring of rats and these effects are relevant to drive firing of CRFR1 or CRFR2 in the PVN neurons and LC NE neurons.



Design

Schematic diagram of the experimental protocol. Pregnant dams in the stressed groups were exposed to IH in a chamber simulating an altitude of 5 km (10.8% O2, 54.02 kPa) (IH), R alone (R), or both (IH+R) daily for 4 h from E1 to E21. The control group parameters were set in the same chamber at sea level (20.9% O2, 100.08 kPa) and left undisturbed. All pups were weaned at P21 and reared to the age of P120 without any disturbance, at which time anxiety-like behavior was tested in the EPM.



Methods

Results

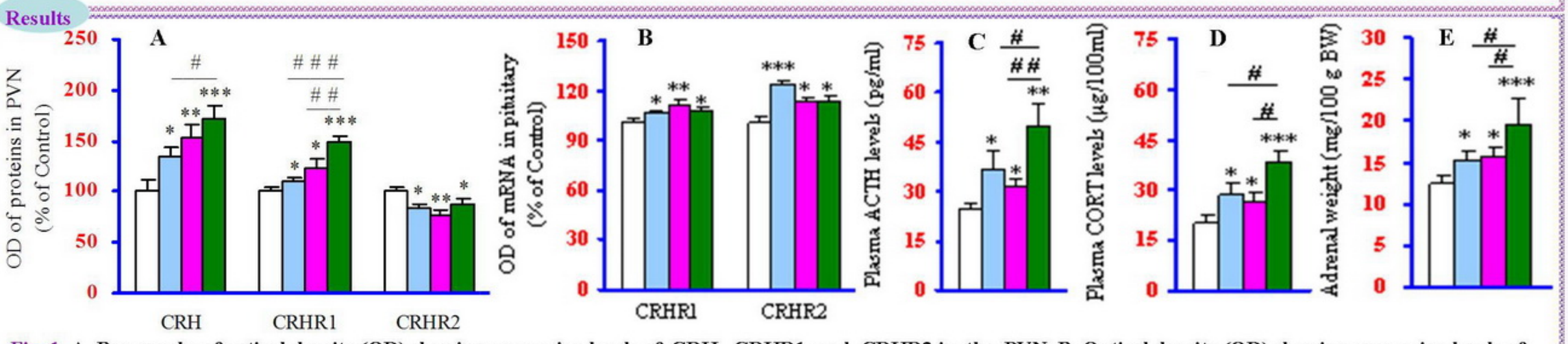


Fig. 1. A, Bar graphs of optical density (OD) showing expression levels of CRH, CRHR1 and CRHR2 in the PVN. B, Optical density (OD) showing expression levels of CRHR1 mRNA and CRHR2 mRNA in anterior pituitary. C, Plasma ACTH levels. D, Plasma CORT levels. E, adrenal weight (mg/100 g BW) , of adult male offspring (P120). N = 6-8. *P <0.05; **P <0.01; ***P <0.001 *vs.* control; #P <0.05 vs. R ; # #P <0.01 *vs.* IH+R.

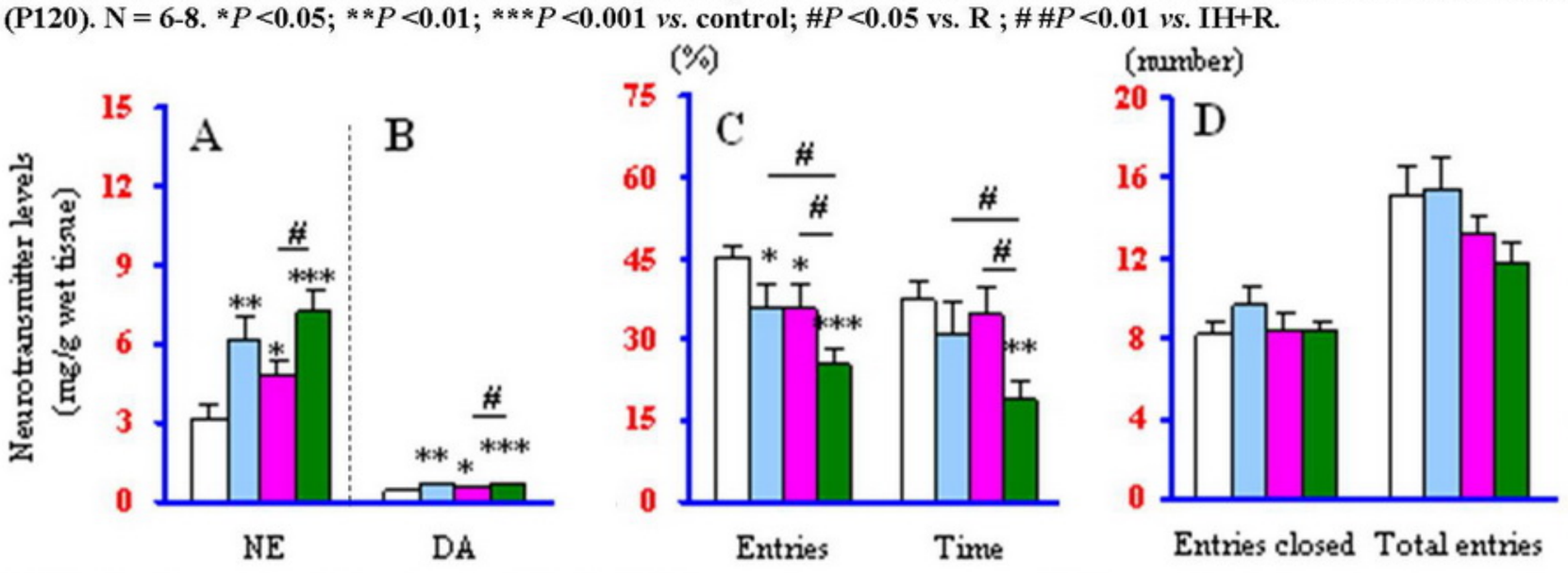


Fig. 2. NE (A) and DA (B) levels in the LC. (C, D) Behavioral parameters in the EPM test of both control and prenatally stressed adult male offspring (P120). N=6–8. *P <0.05; ** P<0.01; *** P<0.001 vs. control; #P<0.05 vs. IH+R.

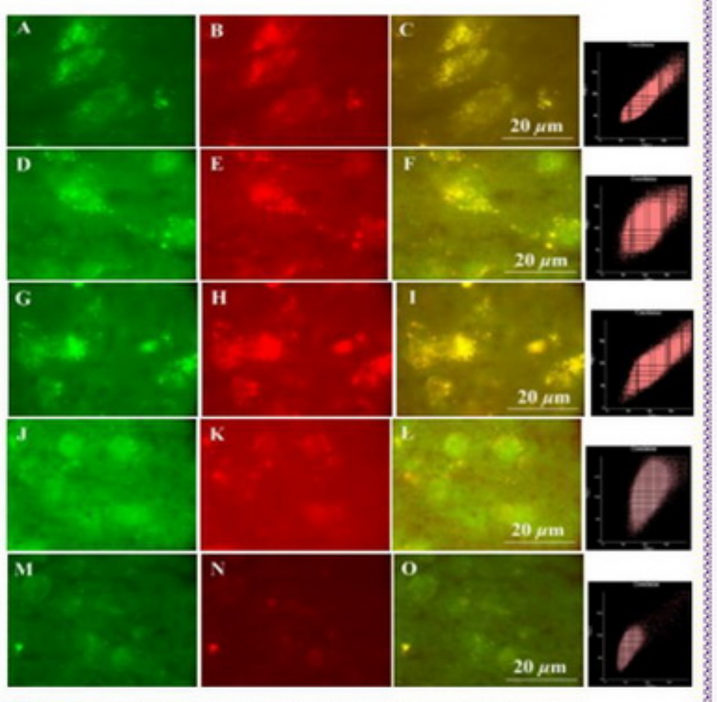


Fig. 3. Confocal photomicrographs for colocalization of CRH and its receptors in the adult rat offspring of mothers that had been exposed to IH+R. Single labeling of CRH/Ucn I/Ucn III neurons (A, D, G, J and M) and CRHR1/ CRFR2 neurons (B, E, H, K and N) in the PVN and LC.

Conclusion

IH, R and IH+R during pregnancy have the potential to activate the CRH-CRHR1-NE neural circuit in the PVN and LC, and to enhance activity of the HPA axis, as well as to induce anxiety-like behavior in adulthood of rats by triggering of PVN CRFR1 which droves the cascade responses of HPA axis and induces an anxiety-liked behavior through activating CRFR1 in PVN and driving NE and DA neurons in LC nuclei. Therefore, physiological (IH) and psychological stress (R) throughout gestation might be a potential risk factor for impaired physiological stress responses and developing anxiogenic behavior in offspring. Colocalization of CRH-CRHR1-CRHR2-NE in the brain may be the neural basis of the behavioral change.

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