

ABSTRACT

Investigation into the biological phenomenon of seasonal reproduction, and its underlying regulatory mechanisms has focused on a wide variety of topics. These include identification of environmental cues which regulate the process and elucidation of the neurobiological pathway for perception and processing of these environmental cues. Photoperiod is the most reliable cue for timing reproduction in seasonal breeding animals. The mechanisms in this regulation are not well understood. Previous studies indicate that in photoperiodic synchronization of the reproductive activity there are involved at least a few regulatory systems, which start with the retina and end at the GnRH pulse generator.

This work is the next step of previous research which shown that the secretion of CO in the eye into the ophthalmic venous blood of the wild boar and pig crossbreed changes depending on season. The aim of this work is to determine by indirect way whether carbon monoxide by counter-current exchange in the perihypophyseal vascular complex may be destination transported to the brain and influence the neural centers of the brain regulating the reproductive processes.

48 sexually mature wild boar and domestic pig crossebred males were used. The experimental animals were assigned to two treatment groups:

In the first experiment the animals received autologous blood plasma infused into the ophthalmic venous sinus for 48 hours with an experimentally induced increase in CO concentration. The identical procedure was performed during the longest days of summer (n=6) and during the shortest days of winter (n=6). Control groups (June n=6; December n=6) received autologous blood plasma infused into the ophthalmic venous sinus for 48 hours.

In the second experiment the animals received autologous blood infused into the ophthalmic venous sinus for 48 hours with an experimentally induced increase in the CO concentration, achieved by two-hour irradiation by LED light. The identical procedure was performed during the longest days of summer (n=6) and during the shortest days of winter (n=6). Control groups (June n=6; December n=6) received autologous blood infused into the ophthalmic venous sinus for 48 hours.

All animals were sampled at 2-hr intervals for 48 hr for LH, FSH and melatonin content in peripheral blood. In order to analyze the effect of CO on *GnRH* and *GnRH-R mRNA* and

gonadotropin subunits (α , $Lh\beta$, $Fsh\beta$) *mRNA* levels, animals in each group were sacrificed after 48h infusion with the experimentally induced increase of concentration of CO. The hypothalamus and pituitary gland were immediately removed, frozen in liquid nitrogen and stored at -80°C until analysis of gene expression.

The peripheral blood samples were used to determine the concentration of hormones using the radioimmunological method. The *GnRH* and *GnRH-R mRNA* and gonadotropin subunits (α , $Lh\beta$, $Fsh\beta$) *mRNA* levels were determined by Real-Time quantitative PCR.

The elevated CO concentration in the blood flowing out of the ophthalmic venous sinus in both experimental groups of males of the wild boar and domestic pig crossbred led to changes in the expression of hypothalamo-pituitary axis genes (*GnRH*, *GnRH-R* and gonadotropin subunits: α , $Lh\beta$ and $Fsh\beta$) during long and short days. There was shown increase in LH secretion in the summer and decrease in LH secretion in the winter. The elevated CO concentration in the blood flowing out of the ophthalmic venous sinus had significant affect on melatonin secretion circadian profile.

To sum up, the results of this study show that light-induced carbon monoxide produced in the eye may be transported in the venous blood and transferred, by counter-current mechanism, to the arterial blood in the perihypophyseal vascular complex and then carbon monoxide can influence the neural centers of the brain regulating the reproductive processes.