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ESTRADIOL-STIMULATED NITRIC OXIDE RELEASE IN NERVOUS TISSUE, VASCULATURE, AND GONADS OF THE GIANT COCKROACH *BLABERUS CRANIIFER**

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The vertebrate system of steroid hormones appears to have been conserved widely throughout the animal kingdom. The sex hormone estrogen, 17-β-estradiol (E_2), long considered to be exclusively a vertebrate hormone, is found also in invertebrates related to reproductive and developmental processes such as spawning, vitellogenesis and molting. These processes are affected by estrogen induced changes at the genomic level and take place at a large time scale. The discovery of surface membrane receptors for E_2 has opened new possibilities for the involvement of estrogen in biological functions other than reproductive. These processes take place within a few seconds to minutes and involve sudden cytosolic calcium transients, activation of adenylate cyclase or activation of phospholipase C (PLC). E_2 can modulate the production of nitric oxide (NO) in endotheliar and other cells. A similar mechanism linking estrogen to cNOS catalized nitric oxide (NO) release is reported herein for the first time in several tissues of the giant cockroach *Blaberus craniifer.* This process has been identified in the brain, nerve cord, vasculature and ovaries. This effect is concentration dependent and is inhibited by tamoxifen an estrogen receptor blocker.

Keywords: Estradiol – estrogen receptors – nitric oxide – invertebrate – insect

INTRODUCTION

Vertebrate steroid hormones and in particular estrogen, E_2, has been found in a number of invertebrates [1, 6–9, 14, 19, 23–26]. These hormones have been found to be involved in reproductive processes such as rate of oviposition, gametogenesis, spawning, vitellogenesis and embryogenesis [6–8, 14, 24, 25]. The majority of these processes involve cellular changes at the genomic level. E_2 can also affect rapid changes in cell function by means of the surface membrane estrogen receptors (ER) [5, 11–13, 15]. ER qualifies as a member of the family of G-protein coupled receptors (GPCR). Upon binding of E_2 to the ER, activation of the G proteins occurs which in turn triggers the intracellular signaling cascades such as cytosolic calcium tran-

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sients, activation of adenylate cyclase or activation of phospholipase C (PLC), affecting biological function.

One of this biological functions is the capacity of E_2 to affect NO release in a variety of cells, both in vertebrates and invertebrates, via a surface membrane ER [2, 4, 17–21, 27]. E_2 affect NO release by either increasing mRNA encoding Nitric Oxide Synthase (NOS) or via the Ca^{2+} dependent constitutive form of NOS. This mechanism is reported herein in the giant cockroach *Blaberus craniifer* where E_2 is shown to generate NO release in a concentration dependent manner in several tissues.

MATERIALS AND METHODS

Animals

The animals, *Blaberus craniifer*, were purchased from Carolina Biological Supplies kept in cages and fed water and food (Purina Chow) *ad libitum*. Prior to dissection the animals were kept on ice for two minutes. After the tissues were dissected they were place in five hundred microliters of Phosphate Buffer Saline (PBS) and immediately assayed for NO.

Nitric oxide measurement

NO release from the tissues was directly measured using an NO-specific amperometric probe (200 μm, World Precision Instruments, WPI, Sarasota, FL). The probe is calibrated daily using the NO donor S-nitroso-N-acetyl-DL-penicillamine. The tissues were exposed to various concentrations of 17β-estradiol and the antagonists tamoxifen and N-ω-nitro-L-arginine methyl ester (L-NAME; Sigma, St. Louis, MO). The data was acquired using the computer-interfaced DUO-18 software (WPI).

RESULTS AND DISCUSSION

As shown in Figures 1–4, the following organs were found to release nitric oxide upon exposure to different concentrations of 17-β-estradiol: brain, nerve chord, dorsal aorta and ovary. The ovaries were found to release the largest amount of NO 25 nM when exposed to 0.4×10^{-7} M 17-β-estradiol whereas the brain only released approximately 1.5 nM NO when exposed to a similar concentration. The effect, as shown in the brain, is concentration dependent and it is blocked by tamoxifen an ER blocker. Similarly we show that the NO release is blocked by L-NAME an indication that the process is catalized by NOS. Although we have not yet ascertained its presence in these tissues, it is surmised that this process is affected via an ER cell surface membrane receptor. Its absence from mRNA extracts of the insect's brain may be due to the existence of an ER different from ERα and ERβ. An alternate ER has been pro-

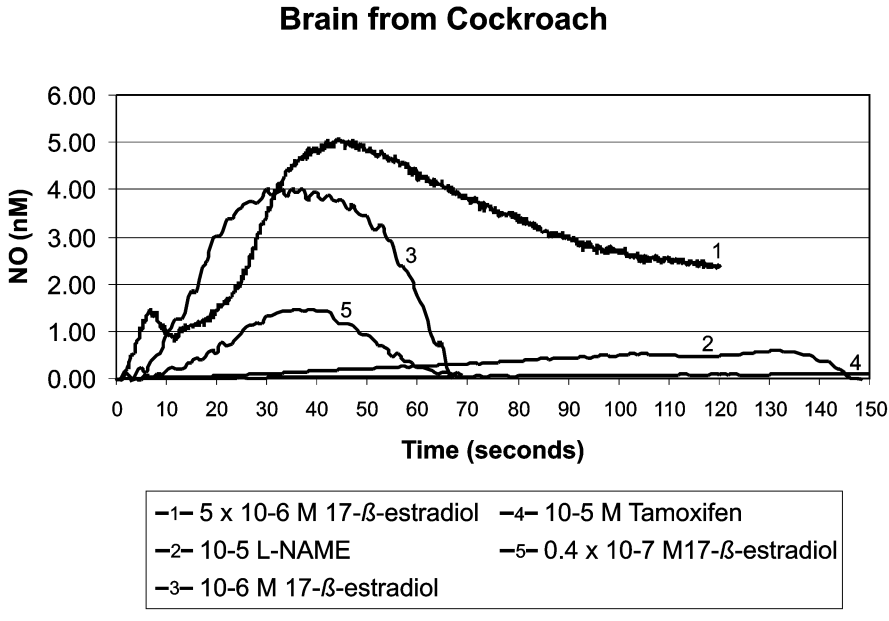

Fig. 1. Nitric oxide release from the brain

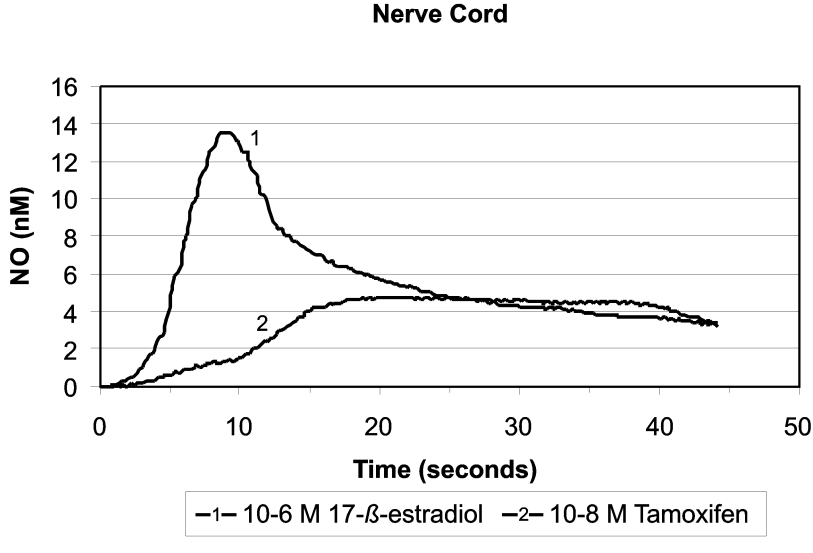

Fig. 2. Nitric oxide release from the nerve cord

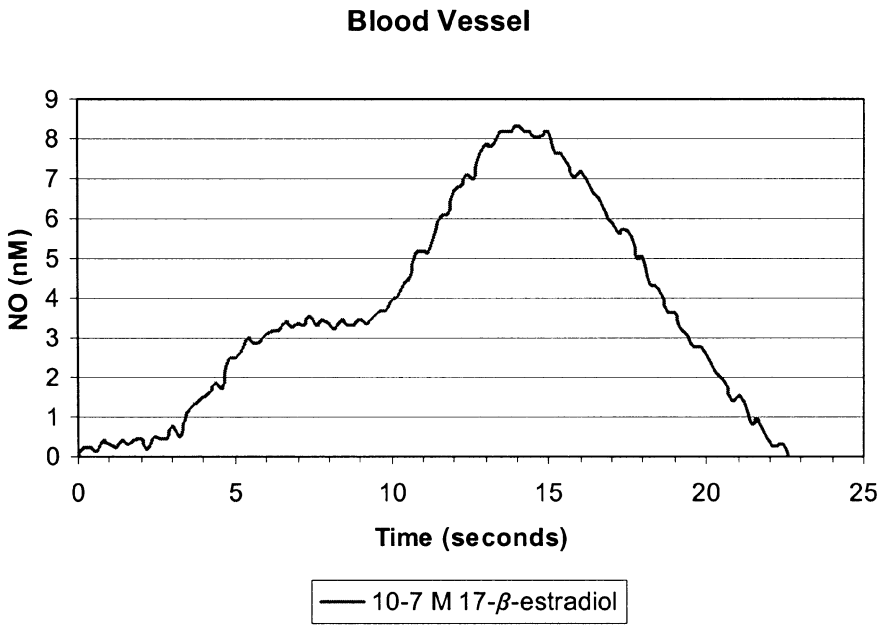

Fig. 3. Nitric oxide release from the blood vessel

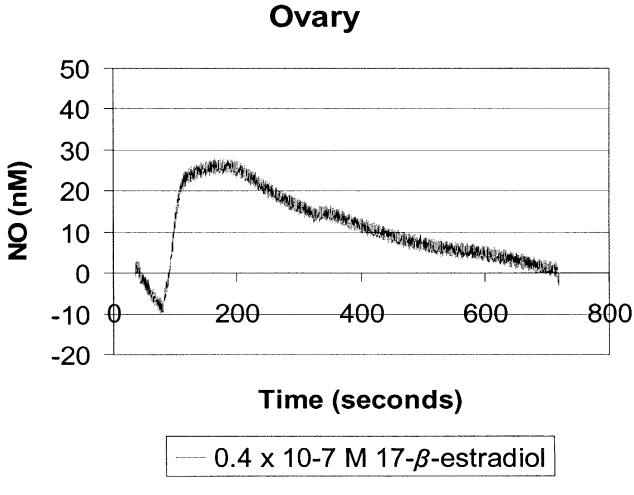

Fig. 4. Nitric oxide release from the ovary

posed also for the CNS of mammals [5]. The binding of E_2 to the receptor affects a cytosolic transient rise in calcium concentration which in turn activates cNOS to catalize the NO release. This process has already been described for human endothelial cells, human granulocytes and in the pedal ganglia of the blue mussel *Mytilus edulis* [17–21]. E_2 has also been shown to affect an increase in expression of eNOS in pulmonary and thoracic aorta of rats [3, 4] and an increase in calcium-dependent translocation of eNOS to the cell membrane of bovine aortic endothelial cells [2]. The inducible isoform of NOS (iNOS) is upregulated upon exposure to E_2 in macrophages [27]. E_2 affected non-genomic effects are very diverse and their biological meaning is beginning to be understood. It has been proposed that its biological function in the cardiovascular and immune systems is to modulate blood vessel tonality and to decrease leukocyte activation and transendothelial migration [3, 4]. In the nervous system NO acts as a neurotransmitter and it has been shown to modulate the activity of both the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis in rats [10, 16, 22]. The biological meaning of the mechanism described in this poster is presently being investigated.

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