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Research report

# Estrogen reduces the severity of autonomic dysfunction in spinal cord-injured male mice

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**Abstract**

Autonomic dysreflexia is an autonomic behavioural condition that manifests after spinal cord injury (SCI) and is characterized by acute, episodic hypertension following afferent stimulation below the level of the injury. Common triggers of autonomic dysreflexia include colorectal distension (CRD), and various somatic stimuli. The development of autonomic dysreflexia is dependent, in part, upon the degree of intraspinal inflammation and the resultant spinal neuroplastic changes that occur following SCI. 17β-estradiol (E) has neuroprotective, anti-inflammatory and smooth muscle relaxant properties, and is therefore a candidate drug for the treatment and/or prevention of autonomic dysreflexia. Autonomic dysreflexia was assessed in adult male mice treated with E. We investigated whether E could be acting centrally by altering: (1) the size of the small diameter primary afferent arbor, (2) the degree of microglia/macrophage infiltration at the site of the injury, or (3) the amount of fibrous scarring present at the injury site. To determine whether E could be working through uncoupling protein-2 (UCP-2), a protein involved with inflammation and regulated by estrogen in some tissues, autonomic dysreflexia was assessed in E-treated adult male mice lacking UCP-2 (UCP-2 KO). 17β-estradiol was equipotent at reducing autonomic dysreflexia in both UCP-2 KO and WT mice following CRD but not tail pinch. We have shown that E reduces autonomic dysreflexic responses to visceral but not somatic stimulation in male mice independent of the size of the primary afferent arbour, the degree of chronic inflammation, and the presence of UCP-2.


*Keywords:* α-CGRP; Estrogen; Spinal cord injury; UCP-2

## 1. Introduction

Traumatic spinal cord injury (SCI) occurs at an incidence ranging from approximately 10–60/1 million people annually [1]. Given that the majority of people afflicted with SCI are injured at a young age and that medical and rehabilitative therapies have improved survival time, the prevalence of SCI is large [1,52]. The general public is aware of the motor disturbances seen in people with SCI (i.e. paraplegia). Behavioural consequences of SCI not commonly thought of by non-spinal cord-injured people include impaired micturition [57], bowel function [14], sexual function [18], and autonomic control of the cardiovascular system [15,31]. Much of the present research evaluating therapies for SCI in rodents is aimed at improving sensorimotor behaviour [47,73]. However, a recent survey indicated that many spinal cord-injured people believe that improvement in bowel and bladder function, sexual function and cardiovascular control would dramatically improve their quality of life [3]. Consequently, there is a need to develop therapies for autonomic behavioural disturbances such as autonomic dysreflexia.

Autonomic dysreflexia is a potentially life-threatening condition occurring in up to 90% of people with severe SCIs at or above the level of the sixth thoracic spinal cord segment (T6) (as cited by Ref. [72]). Autonomic dysreflexia manifests as an acute, severe, uncompensated elevation in systemic arterial blood pressure in response to somatic and/or visceral stimulation below the level of the injury. A variety of hypotheses pertaining to the pathophysiology of autonomic dysreflexia have and/or are being examined [32,34,35,37,48,49,58]. Of particular interest, is the finding that intraspinal inflammation results in an increased pro-

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duction of nerve growth factor (NGF) that leads to an increase in the size of the dorsal horn primary afferent arbor which, in turn, is associated with increased severity of autonomic dysreflexia [9,12,34–36,58]. Reducing the severity of intraspinal inflammation, and presumably the production of NGF, results in the amelioration of autonomic dysreflexia [23].

17β-Estradiol, the most abundant form of estrogen in the body, has been shown to be neuroprotective and therapeutic in various models of central nervous system (CNS) disease where inflammation and immune-mediated processes predominate [6,27,30,38,42,43,55,60,63,65,66,74,76]. 17β-Estradiol exerts its neuroprotective effects, in part, by acting as an anti-inflammatory agent and an anti-oxidant [10,46,59,69,70]. The detailed mechanisms by which 17β-estradiol acts as an anti-inflammatory agent, however, are incompletely understood. There is evidence that 17β-estradiol might affect inflammatory processes by altering mitochondrial uncoupling protein-2 (UCP-2) expression in some tissues [11,56]. Uncoupling protein-2 is one of five members of the uncoupling protein family and, as its name implies, uncouples mitochondrial respiration. A consequence of uncoupling mitochondrial respiration is that oxygen-derived free radicals are reduced. Mortality rates in mice lacking UCP-2 following infection with the protozoal organism *Toxoplasma gondii* are reduced because of improved toxoplasmacidal activity of macrophages due to enhanced free radical production [5]. Further, mice over-expressing UCP-2 are resistant to brain trauma and stroke [44]. With respect to the spinal cord, UCP-2 has been located predominantly within primary afferents of the spinal cord [25]. It seems reasonable to predict that 17β-estradiol may exert, in part, some of its anti-inflammatory effects through UCP-2.

In addition to 17β-estradiol's anti-inflammatory/immunomodulatory effects, 17β-estradiol also has smooth muscle relaxant properties which may help to reduce the incidence of autonomic dysreflexia by improving poor gastrointestinal (GI) transit times and by increasing colonic compliance [17]. For example, some spinal cord-injured patients are susceptible to bowel obstruction, and subsequently autonomic dysreflexia, because of poor GI transit times and reduced colonic compliance [19,40,45].

Considering that (1) the development of autonomic dysreflexia is dependent, in part, upon the degree of intraspinal inflammation following SCI, (2) 17β-estradiol reduces inflammation, (3) 17β-estradiol has been shown to improve hind limb motor function following SCI [76], (4) 17β-estradiol has smooth muscle relaxing properties, and (5) there is evidence to suggest that slightly less women than men may be affected by autonomic dysreflexia early-on in the recovery period following SCI [13], we set out to determine whether 17β-estradiol would alter the development of autonomic dysreflexia in a mouse model of SCI. Further, we behaviourally investigated whether the potentially beneficial effects of 17β-estradiol were dependent upon UCP-2 by using a UCP-2 knock-out (UCP-2 KO) mouse. We hypothesized that the severity of autonomic dysreflexia would be less severe in animals treated with 17β-estradiol. Further, if 17β-estradiol was acting centrally (spinally), we predicted that animals would have reduced autonomic dysreflexic responses following both somatic and visceral stimulation and that the size of the CGRP-labeled primary afferent arbor, and the amount of microglia/macrophage infiltration would be less in animals treated with 17β-estradiol. Also, if 17β-estradiol had any beneficial effects, and if these effects were UCP-2 dependent, we predicted that amelioration of autonomic dysreflexia would be seen in 17β-estradiol-treated WT but not 17β-estradiol-treated UCP-2 KO animals.

## 2. Materials and methods

All procedures carried out in this study were approved by the University of Prince Edward Island Animal Care Committee and were conducted according to the guidelines outlined by the Canadian Council on Animal Care.

### 2.1. Experimental animals

Sixty-one, three- to four-month-old, B6; 129-UCP2$^{tm1Lowl}$ (UCP-2 KO) and B6; 129 (WT) male mice were used for this study. Details regarding the development of these mice are described elsewhere [77]. B6; 129-UCP2$^{tm1Lowl}$ mice have a targeted (knock-out) mutation of the UCP-2 gene between introns 2 and 7. All animals used were derived from C57BL/6J mice and a 129S4/SvJae ES cell line. Heterozygous animals were mated to generate WT, UCP-2 KO, and heterozygous offspring. Animals were bred and raised in the Animal Care Unit of the Atlantic Veterinary College (AVC). All animals were weaned and sexed at 1 month of age and were genotyped as previously described [77]. Mice were housed together, unless fighting occurred, with 12-h light:12-h dark photoperiod in the Animal Care Unit of the AVC. Animals were randomly assigned to a 17β-estradiol-treated ($n=7$ WT; $n=7$ UCP-2 KO) or placebo-treated ($n=8$ WT; $n=7$ UCP-2 KO) group for measuring autonomic dysreflexia, and CGRP and cd11b immunoreactivity.

Animals not used for autonomic dysreflexia measurements ($n=32$) were randomly assigned to 17β-estradiol or placebo treatment groups for determining serum concentration of 17β-estradiol at: (1) 1 week following 17β-estradiol implantation ($n=4$ WT; $n=4$ UCP-2 KO); and (2) 2 weeks following either sham ($n=6$ WT; $n=6$ UCP-2 KO) or spinal cord transection ($n=6$ WT; $n=6$ UCP-2 KO) (note: 3 weeks following 17β-estradiol or placebo pellet implantation).

### 2.2. 17β-estradiol administration

Animals were anesthetized with 1.5% isofluorane (IsoFlo® Abbott Laboratories, Ltd., Saint-Laurent, Que., Canada) in oxygen administered nasally. One pellet containing 0.5 mg 17β-estradiol (Innovative Research of America, Sarasota, FL, USA) designed for constant rate release for 3 weeks or one placebo pellet (containing 0 mg 17β-estradiol) (Innovative Research of America, Sarasota, FL, USA) was subcutaneously implanted over the animal's right scapula. The pellet dose was chosen based on the manufacturer's recommendation so as to achieve physiological serum 17β-estradiol concentrations. Upon completion of the procedure, animals were allowed to recover and were returned to the Animal Care Unit.

### 2.3. 17β-estradiol concentration determination

Animals were deeply anesthetized with sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Ont., Canada). A laparotomy was performed and blood was collected from the abdominal aorta. Blood was allowed to clot and serum was collected following centrifugation. Serum from each animal was stored at −80 °C. 17β-Estradiol serum concentration measurements were made using an ELISA (Calbiotech, Inc., Spring Valley, CA, USA). The lower and upper limits of detection for this test were 1 and 1000 pg/mL, respectively. When 17β-estradiol concentrations exceeded the upper limit of the ELISA, serum was diluted by 50%, the ELISA was repeated, and the concentration was determined by accounting for the dilution factor. The ELISA was performed according to the manufacturer's instructions.