

Research report

Progesterone treatment inhibits the inflammatory agents that accompany traumatic brain injury

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Abstract

Progesterone given after traumatic brain injury (TBI) has been shown to reduce the initial cytotoxic surge of inflammatory factors. We used Western blot techniques to analyze how progesterone might affect three inflammation-related factors common to TBI: complement factor C3 (C3), glial fibrillary acidic protein (GFAP), and nuclear factor kappa beta (NFκB). One hour after bilateral injury to the medial frontal cortex, adult male rats were given injections of progesterone (16 mg/kg) for 2 days. Brains were harvested 48 h post-TBI, proteins were extracted from samples, each of which contained tissue from both the contused and peri-contused areas, then measured by Western blot densitometry. Complete C3, GFAP, and NFκB p65 were increased in all injured animals. However, in animals given progesterone post-TBI, NFκB p65 and the inflammatory metabolites of C3 (9 kDa and 75 kDa) were decreased in comparison to vehicle-treated animals. Measures of NFκB p50 showed no change after injury or progesterone treatment, and progesterone did not alter the expression of GFAP. The therapeutic benefit of post-TBI progesterone administration may be due to its salutary effect on inflammatory proteins known to increase immune cell invasion and cerebral edema.

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1. Introduction

Traumatic brain injury (TBI) is a condition with high morbidity and mortality for which there are currently no treatments that improve clinical outcome measures [45]. The initial biomechanical force in trauma causes ionic imbalances, oxidative damage, microglial activation, immune cell invasion, and cytokine release [3,27,52]. Once this destructive process is initiated, the release of pro-inflammatory cytokines further stimulates immune cells to become phagocytic. Activation of immune cells, in turn, triggers the production of free radicals and additional pro-inflammatory compounds such as cytokines, prostaglandins,

extracellular matrix proteases, complement factors, cell adhesion molecules, and inducible nitric oxide synthase [3,38,43]. The expression of pro-inflammatory factors then attracts immune cells to cerebrovascular surfaces, where they increase vasopermeability, immune cell invasion, and further cytokine generation in the CNS [52]. Regardless of which factors initiate the injury cascade, the inhibition of inflammation may reduce cell death, gliosis, and edema.

The rodent model of medial frontal cortex (MFC) impact injury presents many functional aspects of human head injury, including cognitive and sensorimotor deficits [8,25,40,47,49,57]. Research has shown that progesterone treatment given after MFC injury can reduce these behavioral impairments in rats. Progesterone also reduces edema [47,61], necrosis [51], apoptosis [13,14], blood–brain barrier compromise [46], and the mediators of inflammation [1,2]. In

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this study, we look at some of the cellular mechanisms mediating progesterone's neuroprotective effects with the aim of learning more about how functional recovery occurs.

We now know that progesterone can act in several ways. First, the hormone and its metabolites bind to several cellular receptors and alter their activity. For instance, progesterone acts both as a sigma-1 receptor antagonist (indirectly modulating the NMDA receptor [39]) and as a GABA_A receptor agonist (through conversion to its 3 α -pregnanolone metabolites [62]). Both these actions reduce neuronal excitatory tone and down-regulate excitotoxicity after brain injuries [23]. Though not a free radical scavenger, progesterone can inhibit oxidative damage in the CNS [19,48].

Excitotoxic by-products of oxidative insults trigger an inflammatory immune response by releasing cytokines and other inflammatory factors that contribute to brain edema and neuronal loss. We hypothesize that progesterone treatment reduces neural injury and cerebral edema after TBI by interrupting the inflammatory cascade [36,54,55].

As an anti-inflammatory agent, progesterone can serve as a ligand-gated factor known to inhibit C3 transcription [7,37]. Recently, this receptor/ligand complex has been shown to inhibit the activity of the pro-inflammatory transcription factor, nuclear factor kappa B (NF κ B) [30]. NF κ B is an upstream regulator of inflammation which activates TBI-induced inflammatory cytokines like tumor necrosis factor alpha (TNF α), interleukin 1beta (IL-1 β) [18], C3, and GFAP. The interaction of progesterone with the cascade is worth studying in light of the mounting evidence that inflammatory factors [50] TNF α [16,58], IL-1 β [15], NF κ B [42,63], and C3 [9,32,34] all contribute to brain injury pathology [26,27,36].

2. Methods and materials

2.1. Animals

Twenty adult male Sprague–Dawley rats weighing approximately 280–350 g were used as subjects. All procedures involving animals conformed to guidelines set forth in the “Guide for the Care and Use of Laboratory Animals” (National Academy of Sciences, 1996) and were approved by the Emory University Institutional Animal Care and Use Committee (IACUC of Emory University protocol #101-99). The rats were handled for at least 5 days before surgery and were individually housed. Food and water were provided ad libitum throughout the experiment, and the animals lived in a reversed 12 h light/12 h dark cycle controlled environment.

2.2. Surgery

MFC injury was produced with a computer-controlled pneumatic impactor device described earlier [25]. Rats were anesthetized using isoflurane (5% induction, 2% maintenance,

700 mm N₂O, 300 mm O₂) and mounted in a stereotaxic device with their heads in a horizontal position. Body core temperatures (37 °C) were maintained with a homeothermic heating blanket system (Harvard Apparatus). Using a SurgiVet™ (model V3304) pulse oximeter, blood SpO₂ was monitored and maintained at levels >90%. Under aseptic conditions, a midline incision was made in the scalp, and the fascia retracted to expose the cranium. A centered, bilateral craniectomy was made 3 mm anterior to bregma using a 6-mm diameter drill. After bone removal, the tip of the impactor was moved, using a stereotaxic device, to AP:3.0; ML:0.0, checked for adequate clearance, retracted to its elevated position, and then lowered 3.5 mm DV to penetrate 2 mm into the cortex. The contusions ($n = 10$) were then made at a velocity of 2.25 m/s with a brain contact time of 150 ms. Sham-operated rats ($n = 10$) were anesthetized, mounted in the stereotaxic apparatus, and had their scalps cut and sutured but were not trephined.

2.3. Progesterone preparation and administration

Based on previous studies determining optimal dose response, all progesterone-treated animals received 16 mg/kg progesterone in 25% 2-hydroxypropyl- β -cyclodextrin (HBC) [20]. The HBC vehicle allowed progesterone to be dissolved in a non-toxic, aqueous solution [3,26,52]. The rats received either progesterone ($n = 10$) or an equal volume of vehicle (HBC; $n = 10$) with the first injection given intraperitoneally (IP) 1 h following injury. Subsequent injections were given subcutaneously at 6 h and 24 h after injury. The experimental groups consisted of sham + vehicle (SV; $n = 5$), sham + progesterone (SP; $n = 5$), lesion + vehicle (LV; $n = 5$), and lesion + progesterone (LP; $n = 5$).

2.4. Fresh tissue

The rats were given an overdose IP injection of Nembutal™ (75 mg/kg) and then decapitated. Brains were quickly extracted, divided at bregma, then sagittally bisected, and the hemispheres sectioned into dorsal and ventral halves. Both frontal dorsal sections, which encompassed the injured area, were then frozen in dry-ice-chilled 2-methylbutane and stored at –80 °C.

2.5. Western blot

Western blot analyses were performed on samples that encompassed both the contused and the peri-contused areas of MFC in the injured rats and the equivalent area in the sham-operates. The tissue from each brain was separately homogenized by Dounce in T-per (Pierce, Rockford, IL) with protease inhibitors (Sigma, P8340) and assayed for protein concentration using a Coomassie protein assay (Pierce). An SDS Laemmli sample buffer was then added to sample aliquots and incubated at 90 °C on a heating block for 10 min. Samples containing 30 μ g of protein were then

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