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Single prolonged stress enhances hippocampal glucocorticoid receptor and phosphorylated protein kinase B levels

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ABSTRACT

Animal models of posttraumatic stress disorder (PTSD) can explore neurobiological mechanisms by which trauma enhances fear and anxiety reactivity. Single prolonged stress (SPS) shows good validity in producing PTSD-like behavior. While SPS-induced behaviors have been linked to enhanced glucocorticoid receptor (GR) expression, the molecular ramifications of enhanced GR expression have yet to be identified. Phosphorylated protein kinase B (pAkt) is critical for stress-mediated enhancement in general anxiety and memory, and may be regulated by GRs. However, it is currently unknown if pAkt levels are modulated by SPS, as well as if the specificity of GR and pAkt related changes contribute to anxiety-like behavior after SPS. The current study set out to examine the effects of SPS on GR and pAkt protein levels in the amygdala and hippocampus and to examine the specificity of these changes to unconditioned anxiety-like behavior. Levels of GR and pAkt were increased in the hippocampus, but not amygdala. Furthermore, SPS had no effect on unconditioned anxiety-like behavior suggesting that generalized anxiety is not consistently observed following SPS. The results suggest that SPS-enhanced GR expression is associated with phosphorylation of Akt, and also suggest that these changes are not related to an anxiogenic phenotype.

1. Introduction

Posttraumatic stress disorder (PTSD) presents symptoms including chronic re-experiencing of the traumatic event, hyperarousal, and avoidant behavior (American Psychiatric Association, 1994), and has been associated with enhanced negative feedback of the hypothalamo–pituitary–adrenal (HPA) axis (Weiss, 2007; Yehuda, 2001) and deficits in extinction retention of aversive memories (Jovanovic et al., 2009; Milad et al., 2008). Exploring neurobiological mechanisms by which traumatic stress exposure results in PTSD is difficult to accomplish in humans but can be explored in animal models. The SPS paradigm, originally developed by Liberzon et al. (1997), models certain PTSD symptoms, such as hyperarousal (Khan and Liberzon, 2004; Kohda et al., 2007), and certain PTSD characteristics, such as enhanced HPA-axis negative feedback (Liberzon et al., 1997, 1999), and enhanced contextual fear conditioning (Armario et al., 2008; Yamamoto et al., 2009). In addition, SPS also impairs the retention of a previously extinguished aversive memory (Knox et al., 2012a) and in some studies is reported to produce increased anxiety-like behaviors (Peng et al., 2010; Wang et al., 2010b).

One emerging neurobiological mechanisms by which traumatic stress exposure results in PTSD symptoms is glucocorticoid alterations in individuals with PTSD (Yehuda, 2009). Interestingly, SPS consistently enhances glucocorticoid receptor (GR) protein levels and mRNA expression in the hippocampus (Liberzon et al., 1999; Wang et al., 2009). This effect has been linked to and may directly contribute to SPS-induced phenotypes (Knox et al., 2012b). To investigate the mechanism of GR overexpression and to validate our model with respect to those previous studies, GR protein

Abbreviations: PTSD, posttraumatic stress disorder; SPS, single prolonged stress; EPM, elevated plus maze; OF, open field; GR, glucocorticoid receptor; Akt, protein kinase B; pAkt, phosphorylated Akt; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of Rapamycin; GSK3, glycogen synthase kinase 3; S473, serine residue 473; T308, threonine residue 308; MAPK, mitogen-activated protein kinases; ERK, extracellular signal-related kinase.

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levels were examined in the hippocampus and amygdala. These brain regions were selected because they are sensitive to traumatic stress (McEwen, 2007), critical for proper memory management as well as the behavioral expression of anxiety (Bannerman et al., 2004), and the pathophysiology of PTSD (Bremner et al., 2008).

While SPS-enhanced GR expression is known to contribute to SPS-induced phenotypes, the molecular mechanisms by which GRs mediate SPS-induced phenotypes are unknown. One of the non-genomic mechanisms by which GRs alter neural function is by modulating phosphorylation of kinases and phosphatases (Stahn and Buttgereit, 2008). Akt is a serine/threonine protein kinase that is widely expressed in emotional circuits such as the hippocampus and amygdala, and is phosphorylated (pAkt) at serine residue 473 and threonine residue 308 to produces downstream signaling events (LoPiccolo et al., 2008; Song et al., 2005). Akt has been previously linked to changes in generalized anxiety. For example, previous reports suggest that pAkt has a role in mediating drug-induced and stress-induced changes in anxiety-related behavior (Hauger et al., 2012; Perrine et al., 2008), and it is believed that the regulation of mood and anxiety is through its action on downstream targets (Gould and Manji, 2005). Recently, mice with high trait anxiety show enhanced levels of pAkt (Yen et al., 2012). Alternatively, pAkt has also been associated with trauma-like stress. For example, in another model of PTSD, stress-susceptible mice have increased pAkt levels in the hippocampus and amygdala (Dahlhoff et al., 2010). The increase in pAkt has also been observed in rats exposed to acute restraint and tail shock stress (Yang et al., 2008) and chronic immobilization stress (Lee et al., 2006). Therefore, Akt has been linked to changes in both anxiety and stress, suggesting it may be changed after SPS in brain regions and circuitry that govern mood and emotion. However, the question remains as to whether Akt-related changes are associated specifically with SPS, i.e. GR-driven, or related more to enhanced anxiety after SPS.

While trauma-specific fear is one of the key characteristics of PTSD, generalized (or unconditioned) anxiety is not specific to PTSD, although the two are often comorbid. However, there have been reports in the literature of SPS producing general anxiety-like behavior in rats. These findings vary across studies using SPS. One study did not find unconditioned anxiety-like behavior after SPS (Harvey et al., 2006), while others observed it only after restress (Brand et al., 2008; Harvey et al., 2004; Liberzon et al., 1997), after other modifications in the procedure, such as the addition of a shock stimulus preceding SPS (Wang et al., 2010a), or when SPS occurred during late adolescence (Imanaka et al., 2006) when animals are more susceptible to stressful events (Romeo and McEwen, 2006). This raises the question of the specificity of potential Akt changes and how they relate to specific SPS behavioral effects or to a general increase in anxiety like behaviors.

The main goal of this study was to determine if SPS-enhanced GR expression accompanies increases in pAkt levels in the hippocampus and amygdala. We hypothesized that GR levels would be increased following SPS and that an increase in pAkt levels would accompany this effect. In addition, the current study examined the specificity of Akt and GR changes in relationship to SPS-induced anxiety-like behavior.

2. Materials and methods

The Guide for the Care and Use of Laboratory Animals 7th edition (National Academy Press, Washington, DC) was followed and all experimental procedures were approved by the Institutional Animal Care and Use Committee at Wayne State University and in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care.

2.1. Animals

Male Sprague-Dawley rats ($N = 32$; Charles River Laboratories, Portage, MI) weighing approximately 225-250 g upon arrival were pair-housed in standard microisolator rat (home) polycarbonate cages (45 cm × 26 cm × 21 cm) with bedding. Animals were acclimated to the vivarium for 5-7 days before experimentation during which time the animals were weighed and briefly handled daily. Animals were allowed food and water ad libitum in their home cages and housed on a 12 h light–dark cycle with lights on at 7 AM. Temperature (~24 °C) and humidity (35-40%) were controlled in the vivarium and behavioral testing laboratory.

2.2. Single prolonged stress (SPS)

Half of the animals were exposed to the SPS paradigm as previously described (Knox et al., 2010). Rats were restrained for 2 h in Perspex© restrainers (Plas Labs, Inc., Lansing, MI). This was immediately followed by 20 min of group forced swim ($n = 8$ per swim) in 24 °C water in an approximately 75 l tub (diameter = 45 cm) with a water depth of 28 cm. After the group swim, animals were briefly towel dried and allowed a 15 min recuperation period in new home cages with fresh bedding. Following recuperation, animals were placed in an empty standard rat cage with a wire mesh floor under which two petri dishes filled with diethyl ether anhydrous (50 ml poured into open dishes) were placed. Rats were exposed to ether until loss of consciousness, which took approximately 3-5 min and was as observed visually and confirmed by tail or paw pinch. Rats were placed back into their fresh home cages. The other half of the animals (i.e. the control animals) were briefly handled in a separate area of the laboratory during the time of the SPS procedure, and then placed into home cages with fresh bedding. All animals were returned to the vivarium and housed for 7 days without disturbance other than to check on health status and replenish food and water if necessary.

2.3. Experiment 1 – locomotor activity

Locomotor activity was measured overnight in a group of SPS ($n = 4$) and control ($n = 4$) rats to determine if SPS increases locomotor activity during the onset or end of the dark cycle. Locomotor activity was measured by an automated monitoring system (Digiscan DMicro, Accuscan Instruments, Columbus, OH), which consists of 16 parallel infrared emitter/detector photocells mounted on a metal assembly into which a standard home cage without bedding was placed. Data were recorded by Accuscan software installed on a PC computer linked to the activity monitors, and activity was measured as total photocell beam break counts. Animals were placed individually into a homecage environment with free access to food and water while locomotor activity was monitored overnight from 5 PM to 9 AM (16 h total). Animals were tested for baseline activity for three consecutive nights prior to SPS exposure, and then re-tested three nights following the 7 day undisturbed period after SPS (Fig. 2A). In another group of SPS ($n = 4$) and control ($n = 4$) rats, general locomotor behavior was measured during the day in an OF to determine if SPS increases general locomotor behavior in a novel environment during the light cycle. The OF consisted of a large testing chamber made of black Plexiglas material with no lid and matte black floor (80 cm × 80 cm × 36 cm; Formtech Plastics, Oak Park, MI). Animals were started in the center of the OF and spontaneous activity was recorded for 10 min with a digital CCD camera that was connected to a PC computer installed with an automated tracking software package (Ethovision 6.1, Noldus, Inc., Leesburg, VA). The total distance traveled was used as an index to assess behavioral activity. Animals were tested two consecutive