Psychoneuroendocrinology (2015) 53, 136—147

Available online at www.sciencedirect.com

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In a rat model of panic, corticotropin responses to dorsal periaqueductal gray stimulation depend on physical exertion

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Received 5 October 2014; received in revised form 29 December 2014; accepted 29 December 2014

KEYWORDS
Panic attack;
Periaqueductal gray matter;
Stress;
Corticotropin;
Corticosterone;
Prolactin

Summary Panic disorder patients are exquisitely and specifically sensitive to hypercapnia. The demonstration that carbon dioxide provokes panic in fear-unresponsive amygdala-calcified Urbach-Wiethe patients emphasizes that panic is not fear nor does it require the activation of the amygdala. This is consonant with increasing evidence suggesting that panic is mediated caudally at midbrain's dorsal periaqueductal gray matter (DPAG). Another startling feature of the apparently spontaneous clinical panic is the counterintuitive lack of increments in corticotropin, cortisol and prolactin, generally considered 'stress hormones'. Here we show that the stress hormones are not changed during DPAG-evoked panic when escape is prevented by stimulating the rat in a small compartment. Neither did the corticotropin increase when physical exertion was statistically adjusted to the same degree as non-stimulated controls, as measured by lactate plasma levels. Conversely, neuroendocrine responses to foot-shocks were independent from muscular effort. Data are consonant with DPAG mediation of panic attacks.

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http://dx.doi.org/10.1016/j.psyneuen.2014.12.022
0306-4530/

1. Introduction

Panic disorder (PD) patients are specifically sensitive to both the intravenous infusion of 0.5 M sodium lactate (LAC) and the inhalation of 5—7% carbon dioxide (CO_2). These and other evidence led Klein (1993) to propose that clinical panics are the misfiring of a suffocation alarm system. Klein's 'suffocation false alarm' theory was further supported by the demonstration that panic patients are also hypersensitive to hypoxia (Beck et al., 1999). Remarkably, as well, clinical panics are not accompanied by increases in the secretion of the 'stress hormones' corticotropin (ACTH), cortisol (COR) and prolactin (PRL). Indeed, neither the situationally provoked panic attacks of agoraphobics (Woods et al., 1987) nor the experimentally provoked panics to LAC and CO_2 (Liebowitz et al., 1985; Levin et al., 1987; Woods et al., 1988; Hollander et al., 1989a,b; Sinha et al., 1999) increased stress hormones significantly relative to healthy controls. COR plasma levels actually decreased in 10 panicking subjects exposed to 7% CO_2 (Sinha et al., 1999). Despite a small sample size, Cameron et al. (1987) also did not find any change in stress hormones either at peak or 10 and 60 min after 9 spontaneous panic attacks in 4 hospitalized patients.

Cortisol concentrations were nevertheless increased in the saliva of patients having severe panic attacks (Bandelow et al., 2000). Although the authors were unable to replicate these findings (personal communication to Donald F. Klein), the hypothalamus-pituitary-adrenal (HPA) axis is also activated in human, fear-like, panics marked by palpitations, tremor and sweating, that are devoid of suffocation symptoms. These fear-like panics can be provoked by drugs that either produce anxiety (Kamilaris et al., 1992; Graeff et al., 2005) or stimulate *in vitro* neurons of the paraventricular nucleus of the hypothalamus (PVN) (Kamilaris et al., 1992).

That panic is distinct from fear was shown in a recent article reporting the 35% CO_2 provocation of panic in fear-unresponsive Urbach-Wiethe disease patients with extensive bilateral calcifications of the amygdala (Feinstein et al., 2013). Consequently, the authors concluded that panic is mediated 'at the brainstem' in spite of the established role of the amygdala in fear and anxiety of both humans and animals. Indeed, pre-clinical data from our laboratory (Schimitel et al., 2012, 2014) showed that the dorsal half of midbrain's periaqueductal gray matter (DPAG) harbors a hypoxia-sensitive suffocation alarm system which activation in humans might both trigger a spontaneous panic attack and render the subject hyperresponsive to CO_2. These findings were corroborated by c-fos immunohistochemistry data showing the activation of both the nucleus of the solitary tract (NTS) and the DPAG in rats that developed escape reactions to 8% hypoxia (Casanova et al., 2013). Overall, these data endorse extensive research suggesting the DPAG mediation of panic (Deakin and Graeff, 1991; Schenberg et al., 2001, 2014; Mobbs et al., 2007; Quintino-Dos-Santos et al., 2014).

The DPAG mediation of panic was also supported by the lack of increase in both ACTH and PRL plasma levels 5 and 15 min after the panic-like behaviors produced by 1-min electrical stimulations of DPAG (Schenberg et al., 2008). However, further analyses showed that corticosterone (CORT) baseline plasma levels were significantly increased (285.2 ± 8 ng/mL) one week after the electrode implantation (L.C. Schenberg, unpublished results). Accordingly, the lack of ACTH responses in our previous study might have been due to the CORT increased level inhibition of HPA axis in rats recently implanted with both an electrode (7 days prior to testing) and an indwelling catheter (2 days prior to testing). Additionally, a recent study of Lim et al. (2011) reported a conspicuous increase (160%) in CORT plasma level 30 min after a DPAG-evoked 1-min explosive flight bout ('running with aimless direction') in 4-week surgery-recovered rats. Consequently, these authors argued that the lack of stress hormone responses of our previous study (Schenberg et al., 2008) was due either to the HPA axis inhibition in rats recently operated or to the blood sampling timing which missed hormone responses. Conversely, such conflicting data might be explained by the different degrees of physical effort during DPAG-evoked flight behaviors in arenas of 0.3 m^2 (Schenberg et al., 2008) and 1 m^2 (Lim et al., 2011). That physical effort is relevant in DPAG-evoked neuroendocrine responses was already acknowledged by Schenberg et al. (2008) report of a moderate (83%) though non-significant increase in ACTH plasma levels following the exhausting effort of DPAG-evoked repetitive flight bouts.

Accordingly, the present study examined whether the DPAG-evoked increases in ACTH (Schenberg et al., 2008) and CORT (Lim et al., 2011) secretions were due to the physical exertion during escape behaviors. This problem was approached both analytically, by correlation of neuroendocrine responses and muscular exertion as measured by LAC plasma levels, and experimentally, examining the DPAG-evoked neuroendocrine responses of freely-moving rats stimulated in a small compartment that prevented the flight behavior. Furthermore, the HPA axis responsiveness of 4-week surgery-recovered rats was assessed examining the neuroendocrine responses to two environmental stressors, i.e., a 30-min exposure to a brightly-lit (180 lx) open-field or a foot-shock of the same duration (1 min) as the intracranial stimulus.

2. Methods

2.1. Animals

Male albino Wistar rats ($n = 100$) were bred in the stress-controlled animal facility of our laboratory. The facility was a restricted room with periodic renewal of the air and controlled conditions of temperature (23—25 °C), sound (46 dB white noise) and light (12 h light/dark cycle, lights on at 6:00 am). As adults, rats were kept in groups of 2—4 subjects in polypropylene boxes (60 cm × 50 cm × 22 cm) with food and water *ad libitum* and wood shave bedding. This study was approved by the local Committee on the Ethical Use of Animals (CEUA 099/2011) and complied with EU Directive 2010/63/EU on the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).

2.2. Electrode implantation

Except for the unimplanted groups of Experiment-1, all rats were implanted with electrodes aimed to the DPAG. Rats