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EVIDENCE OF A SUFFOCATION ALARM SYSTEM WITHIN THE PERIAQUEDUCTAL GRAY MATTER OF THE RAT

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Abstract—Dyspnea, hunger for air, and urge to flee are the cardinal symptoms of panic attacks. Patients also show baseline respiratory abnormalities and a higher rate of comorbid and antecedent respiratory diseases. Panic attacks are also precipitated by infusion of sodium lactate and inhalation of 5% CO_2 in predisposed patients but not in healthy volunteers or patients without panic disorder. Accordingly, Klein [Klein (1993) Arch Gen Psychiatry 50:306–317] suggested that clinical panic is the misfiring of an as-yet-unidentified suffocation alarm system. In rats, selective anoxia of chemoreceptor cells by potassium cyanide (KCN) and electrical and chemical stimulations of periaqueductal gray matter (PAG) produce defensive behaviors, which resemble panic attacks. Thus, here we examined the effects of single or combined administrations of CO_2 (8% and 13%) and KCN (10–80 μg, i.v.) on spontaneous and PAG-evoked behaviors of rats either intact or bearing electrolytic lesions of PAG. Exposure to CO_2 alone reduced grooming while increased exophthalmus, suggesting an arousal response to non-visual cues of environment. Unexpectedly, however, CO_2 attenuated PAG-evoked immobility, trotting, and galloping while facilitated defecation and micturition. Conversely, KCN produced all defensive behaviors of the rat and facilitated PAG-evoked trotting, galloping, and defecation. There were also facilitatory trends in PAG-evoked exophthalmus, immobility, and jumping. Moreover, whereas the KCN-evoked defensive behaviors were attenuated or even suppressed by discrete lesions of PAG, they were markedly facilitated by CO_2. Authors suggest that the PAG harbors an anoxia-sensitive suffocation alarm system which activation precipitates panic attacks and potentiates the subject responses to hypercapnia.

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Abbreviations: Bar, Barrington's nucleus; CeA, central amygdala; CUN, cuneiform nucleus; DEF, defecation; DLPAG, dorsolateral periaqueductal gray; DMH, dorsomedial hypothalamus; DMPAG, dorsomedial periaqueductal gray; DPAG, dorsal periaqueductal gray; DR, dorsal raphe nucleus; ED_{50}, median effective dose; EXO, exophthalmus; GLP, galloping; I_{50}, median effective intensity; IMO, immobility; JMP, jumping; KCN, potassium cyanide; LC, locus coeruleus; LPAG, lateral periaqueductal gray; MIC, micturition; MPAG, medial periaqueductal gray matter; NTS, nucleus of the solitary tract; PAG, periaqueductal gray matter; PBA, parabrachial area; PET, positron emission tomography; PH, posterior hypothalamus; PMC, pontine micturition center (or M-region); PMD, premammillary dorsal nucleus; PPT, pedunculopontine tegmental nucleus; RA, nucleus retroambiguus; TRT, trotting; VLPAG, ventrolateral periaqueductal gray; χ^2, chi-square.

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Klein (1993) suggested that clinical panic is bound to the fear of suffocation produced by the misfiring of an as-yet-unidentified suffocation alarm system. This would result in a sudden dyspnea followed by a brief hyperventilation, panic, and the urge to flee. Klein (1993) argued that the false suffocation alarm hypothesis is a consistent explanation of the hypersensitivity of panic patients to sodium lactate and CO_2, of panic attacks during relaxation and sleep, of the increased frequency of panic attacks in the late luteal phase dysphoric disorder and, conversely, its reduction in pregnancy, delivery, and lactation. Indeed, CO_2- and lactate-induced panic attacks remain the best models of clinical panic insofar that they are not precipitated in healthy subjects (Pitts and McClure, 1967; Klein, 1993) or patients with obsessive-compulsive disorder (Griez and Schruers, 1998), social phobia (Liebowitz et al., 1985b) and, less certainly, generalized anxiety disorder (Lapierre et al., 1984). Moreover, whereas CO_2- and lactate-induced panics are blocked by chronic treatment with tricyclic antidepressants (Rifkin et al., 1981; Liebowitz et al., 1985a; Woods et al., 1990; Yeragani et al., 1988; Gorman et al., 1997), β-carboline and yohimbine precipitate panic attacks in healthy subjects that are blocked by low doses of diazepam but not tricyclics (Dorow et al., 1983; Klein, 1993). Recently, Preter and Klein (2008) expanded the suffocation false alarm hypothesis suggesting that the high comorbidity of panic disorder with childhood separation anxiety is related to a dysfunction of an endogenous opioidergic system common to both conditions. Indeed, Preter and collaborators (2011) showed that naloxone-treated healthy volunteers present panic-like respiratory responses to intravenous infusions of sodium lactate. The presumptive link of suffocation alarm system and childhood separation anxiety was further supported by recent studies showing that rats subjected to neonatal mother separation present a sex-dependent facilitation of respiratory responses to both hypoxia (males) and hypercapnia (females) in adult life (Genest et al., 2004, 2007a,b; Dumont et al., 2011).

On the other hand, electrical and chemical stimulations of periaqueductal gray matter (PAG) produce defensive behaviors in animals (Bittencourt et al., 2004; Schenberg et al., 2005) and emotional feelings and symptoms in humans (Nashold et al., 1969; Young, 1989), which resemble panic attacks (Deakin and Graeff, 1991; Jenck et al., 1995; Schenberg et al., 2001). Interestingly, PAG stimulations in humans were also reported to produce smothering

or a "choking feeling referred into the chest" (Nashold et al., 1969; Kumar et al., 1997). Although the PAG neuron systems very often extend beyond the tectospinal fibers usually accepted as the border of the PAG (Holstege, 1991), aversive emotions in humans and defensive responses in animals are both produced by electrical or chemical stimulations of the "dorsal PAG" (DPAG), that is, the region comprising the dorsomedial (DMPAG), dorsolateral (DLPAG), and lateral (LPAG) columns of PAG (Keay and Bandler, 2004) as well as the adjoining areas of medial PAG (MPAG) (Veening et al., 1991).

Although the DPAG-evoked behaviors have been collectively regarded as a model of panic attack, pharmacological studies suggest that galloping is the panic attack best-candidate response (Schenberg et al., 2001). Indeed, whereas the DPAG-evoked galloping was either attenuated or virtually abolished by chronic administrations of panicolytics in doses and regimens alike to those of the therapy of panic disorder, it was hardly affected by clinically ineffective treatments, including both the acute injections of antidepressants and benzodiazepines (Schenberg et al., 2001; Vargas and Schenberg, 2001) and the acute and 10-day injections of buspirone (L.C. Schenberg and L.C. Vargas, unpublished observations). Conversely, PAG-evoked galloping was facilitated by the putative panicogens pentylenetetrazole (Schenberg et al., 2001) and cholecystokinin (Jenck et al., 1995; Bertoglio et al., 2007). The PAG involvement in panic attacks was further supported by recent studies from our laboratory showing that PAG-evoked defensive responses are markedly facilitated in adult rats that were subjected to neonatal mother separation (Quintino dos Santos, 2011).

On the other hand, incidental observations of Franchini and collaborators (Franchini and Krieger, 1993; Franchini et al., 1997) showed that the selective cytotoxic anoxia of chemoreceptor cells by low doses of potassium cyanide (KCN) evokes defensive behaviors in rats. Moreover, c-fos immunohistochemistry studies showed that the PAG is markedly activated by both the repeated administrations of KCN (Hayward and von Reitzenstein, 2002) and the prolonged exposures to CO_2 (Teppema et al., 1997; Berquin et al., 2000). Nevertheless, whereas the KCN produced a widespread activation of PAG, the CO_2 activated caudal districts of the ventrolateral PAG (VLPAG), which on stimulation produces quiescence and hyporeactive immobility, but not overt defensive behaviors (Morgan and Carrive, 2001). Yet, the LPAG was similarly activated by both the KCN and CO_2 (Hayward and von Reitzenstein, 2002; Berquin et al., 2000).

Above evidence suggests that the PAG harbors a suffocation alarm system which dysfunction could precipitate spontaneous panic attacks. Accordingly, here we examined the effects of individual and combined administrations of CO_2 and KCN on spontaneous and PAG-evoked behaviors of rats. Behavioral effects of KCN were also examined before and after electrolytic lesions of PAG.

EXPERIMENTAL PROCEDURES

Animals

Male adult Wistar rats (n=60), weighing between 230 and 260 g, were housed in individual glass walled cages (25×15×30 cm^3) with food and water *ad libitum*. Cages were kept in a temperature controlled room (20–25 °C) and 12-h light/dark cycle (lights on at 6:00 AM). All efforts were made to minimize suffering and the number of animals. Experiments conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, 1996) and were approved by the local committee on the ethical use of animals in scientific research (CEUA-UFES, 004/2009).

Electrodes and surgery

Electrodes were made of a stainless steel wire (0.25 mm o.d.) (California Fine Wire Company, Grover City, USA) insulated throughout except at the cross section of the tip. A non-insulated stainless steel wire served as the indifferent electrode. Electrode implantation was carried out as previously described (Bittencourt et al., 2004).

Implantation of intra-atrial catheter for KCN injections

Rats were anesthetized with chloral hydrate and implanted with a heparin-filled intra-atrial silastic catheter (1-mm diameter) inserted through the right jugular vein. Following implantation, the catheter was tunneled under the skin and exposed at the nape.

CO_2 chamber

The CO_2 chamber was a 50-cm wall height and diameter plexiglas cylinder provided with a lid and rubber seals in the upper and lower rims. The lid had an electric mercury swivel to allow the stimulation of freely moving rats, an exhaustion fan for air renewal following the administration of CO_2, and a smaller fan fixed to its inner surface to promote the homogeneous distribution of CO_2. The chamber was placed in a sound-attenuated temperature-controlled room (22–25 °C) and was connected through a flow valve to a CO_2 cylinder. The CO_2 was administered at vapor pressure, at the rate of 27 L/min, throughout 15 s or 30 s. The chamber was calibrated by means of a gas analyzer (Testoryt, Confor Instruments, São Paulo, Brazil) at intervals of 0.5, 1, 2, 3, 4 and 5 min after the administration of CO_2, with or without the renewal of the air. CO_2 mean concentrations were computed over six administrations of 15 s or 30 s.

Behavioral recording

The frequency and duration of spontaneous and KCN-evoked behaviors in room air and CO_2-enriched mixtures were computed off-line by video analysis. In turn, PAG- and KCN-evoked "threshold responses," that is, the responses emitted with minimally effective currents or doses, were recorded on-line in a binary way (i.e. as emitted or not, irrespective of the response frequency or duration in a single stimulation/injection trial). The rat defensive behaviors were recorded according to a statistically validated ethogram (Bittencourt et al., 2004), as follows:

Exophthalmus (EXO)—The eyes take on a spherical shape due to the eyeball protrusion and fully opening of the eyelid (McHaffie and Stein, 1982).

Immobility (IMO)—Overall behavioral arrest accompanied by the increase in muscle tonus as suggested by the extension of neck and/or limbs and elevation of head, trunk, and/or tail. Except for the visible tachypnea, the rat looks like a "statue" for periods as short as 3 s or lasting the whole stimulation trial (30 s). Tense immobility was invariably accompanied by exophthalmus but not the inverse.