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Dorsal periaqueductal gray matter-evoked panic-like behaviors are markedly inhibited by a low peripheral dose of thyrotropin releasing hormone

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Summary Stimulation of the dorsal periaqueductal gray matter (DPAG) produces defensive behaviors which are reminiscent of panic attacks. Recent evidence from our laboratory showed that DPAG-evoked defensive behaviors are markedly attenuated in short-term methimazole-induced hypothyroidism. It is not clear, however, whether these effects were due to an increase in thyrotropin releasing hormone (TRH), a decrease in thyroid hormones or to the overall effects of hypothyroidism. Accordingly, here we examined whether the peripheral injection of TRH has any effect either on the panic-like behaviors induced by electrical stimulation of DPAG or anxiety-like behaviors of rats exposed to the elevated plus-maze (EPM). Rats whose stimulation of DPAG produced flight responses (galloping or jumping) with intensities below 60 μA were injected with 1 μg/kg TRH (i.p.) and stimulated 10 min after that. The day after, rats were treated with saline and subjected to the same stimulation procedure. Threshold curves were fitted through the logistic model and compared by likelihood-ratio χ^2 tests. TRH and saline effects on EPM performance were appraised in separate groups. Compared to saline-sessions, TRH-injected rats presented thresholds significantly higher for immobility (40%), trotting (33%), galloping (34%), jumping (39%) and exophthalmus (43%). In contrast, TRH had no effects on EPM arm exploration. TRH selective inhibition of DPAG-evoked defensive behaviors adds new evidence that panic attacks may be attenuated by increased levels of this hormone in hypothyroidism.

Electrical and chemical stimulations of dorsal periaqueductal gray matter (DPAG) produce defensive behaviors which have been proposed as a model of panic attack (Gentil, 1988; Deakin and Graeff, 1991; Jenck et al., 1995; Schenberg et al., 2001; Vargas and Schenberg, 2001). Most notably, the DPAG-evoked galloping of the rat was markedly attenuated by chronic administration of clinically effective panicolytics (fluoxetine, clomipramine) given in doses and regimen similar to those employed in the therapy of panic disorder (PD) (Schenberg et al., 2001; Vargas and Schenberg, 2001). Conversely, this response was facilitated by the putative panicogens pentylenetetrazole, yohimbine and cholecystokinin (CCK) (Schenberg et al., 2001; Jenck et al., 1995; Bertoglio et al., 2007). In

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addition, DPAG-evoked responses were barely affected by treatments which are ineffective in PD, including the acute injections of antidepressants and benzodiazepines (Schenberg et al., 2001; Vargas and Schenberg, 2001) and acute and 10-day treatments with buspirone (L.C. Schenberg and L.C. Vargas, unpublished results). As well, recent studies from our laboratory showed that DPAG-evoked defensive behaviors are not accompanied by increases in the secretion of 'stress hormones', corticotropin and prolactin (Schenberg et al., 2008). The latter results are noteworthy in so far that they reproduce the lack of stress hormone responses following panic attacks induced by intravenous infusion of sodium lactate or the patient exposure to a panic-inducing context (Graeff et al., 2005; Schenberg et al., 2008).

Contrarily, electrical and chemical stimulation of ventrolateral periaqueductal gray matter (VLPAG) both inhibits the neuron activity within the DPAG (Lovick, 1991) and produces quiescent behavior and hyporeactivity (Depaulis et al., 1994; Morgan and Carrive, 2001). The VLPAG also harbors the major extra-hypothalamic population of thyrotropin releasing hormone (TRH)-synthesizing neurons (Liao et al., 1988). Most notably, while the TRH seems to be also synthesized in caudal raphe and parapyramidal region of medulla (Yang et al., 1999; Nillni and Sevarino, 1999), the full processing of TRH precursor peptide (prepro-TRH) to TRH was only demonstrated in the paraventricular and preoptic nuclei of hypothalamus and in the VLPAG (Nillni and Sevarino, 1999). Interestingly enough, VLPAG TRH neurons were shown to project to the parafascicular nucleus of thalamus, DPAG, deep layers of superior colliculus and a region bordering the nucleus of Barrington and the locus coeruleus (Mihaly et al., 2001). These areas are remarkable insofar that they are all involved in the control of defensive behaviors. Accordingly, whereas the parafascicular nucleus of thalamus was recently implicated in the emotional processing of pain (Harte et al., 2005), the DPAG and deep collicular layers play a major role in the organization of defensive behaviors, as demonstrated by lesion (Blanchard et al., 1981), electrical or chemical stimulation (Bandler et al., 1985; Dean et al., 1989; Depaulis et al., 1992; Bittencourt et al., 2004, 2005; Schenberg et al., 2005) and c-fos immunostaining studies (Canteras and Goto, 1999; Dielenberg et al., 2001). In turn, the nucleus of Barrington is recognized as the central controller of micturition and defecation responses which have been traditionally associated to fear and anxiety (Blok and Holstege, 1998; Valentino et al., 2000). These findings are in keeping with electrophysiological, track-tracing and c-fos immunostaining evidence showing that the DPAG projects to the nucleus of Barrington, locus coeruleus and neighboring regions (Levine et al., 1990; Ennis et al., 1991; Luppi et al., 1995; Sandkuhler and Herdegen, 1995; Valentino et al., 2000). Pending much further evidence, the above data suggest that VLPAG TRH neurons could exert a tonic inhibitory modulation of DPAG-evoked panic-like behaviors.

As well, the VLPAG TRH system could be modulated by thyroid states, thereby dampening or facilitating panic attacks. As a matter of fact, recent data from our laboratory showed that DPAG-evoked defensive behaviors are markedly attenuated following a 10-day treatment with the anti-thyroid agent methimazole (MTZ) (Siqueira et al., unpublished results). It is not clear, however, whether these effects were due to an increase in thyrotropin releasing hormone (TRH), to a decrease in thyroid hormones, to systemic effects of hypothyroidism, or whatever combination of these effects.

Therefore, the present study examined the effects of peripheral injections of a low dose of TRH either on the panic-like behaviors induced by electrical stimulation of DPAG or anxiety-like behaviors of rats exposed to elevated plus-maze (EPM).

1. Materials and methods

1.1. Animals

Male adult Wistar rats ($n = 72$), weighing 210 ± 20 g (mean $\pm$ S.D.), were housed in individual glass walled cages (25 cm $\times$ 15 cm $\times$ 30 cm) with food and water *ad libitum*. The 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 Brazilian Society of Neuroscience and Behavior's (SBNeC) guidelines on the ethical use of animals in scientific research.

1.2. Electrodes

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. The electrode was soldered to one pin of a miniature socket (BCPT 50, Celis, São Paulo, Brazil). A non-insulated stainless steel wire was connected to the other pin and served as the indifferent electrode.

1.3. Brain surgery

Rats were anesthetized with 400 mg/kg (i.p.) chloral hydrate (Isofar, Rio de Janeiro, Brazil) and fixed on a stereotaxic instrument (David Kopf, Tujunga, USA) with the skull horizontal between bregma and lambda. Following the scalp infiltration with 1% lidocaine plus 0.005% epinephrine (Cristália, São Paulo, Brazil), the bone over the lambda was abraded with a diamond-coated dentistry drill (KG Sorensen, São Paulo, Brazil) and removed with thin forceps so as to expose the sinus. The electrode insertion was facilitated by a small incision of the dura. Whenever necessary the sinus was gently pushed with the electrode itself, thereby allowing its penetration to the aimed site in the DPAG (−7.6 mm AP and 0.4 mm LAT in relation to bregma, and −4.4 mm VERT in relation to cortex surface). The prosthesis was fixed with autopolymerizing resin and 4 stainless steel screws fixed to the skull. At the end of surgery the electrode pins were protected by a plastic tube set around them. Finally, rats received 24,000 I.U. of penicillin-G benzathine (i.m.) and were placed over a heated platform until their full recovery.

1.4. EPM

The apparatus was a white formica-covered plus-shaped wooden maze made up of 2 enclosed arms (50 cm $\times$ 10 cm $\times$ $\times$ 40 cm) perpendicular to 2 open arms (50 cm $\times$ 10 cm).