

PARTICIPATION OF THE CENTRAL SEROTONERGIC PATHWAY IN THE ENDOTOXIN-STIMULATED HYPOTHALAMO-PITUITARY-ADRENAL AXIS FUNCTION

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Summary The aim of the present study was to determine whether the central serotonergic pathway (5HT) plays any stimulatory/inhibitory role in endotoxin-stimulated hypothalamo-pituitary-adrenal (HPA) axis function in the male rat. For this purpose, animals inhibited or not of central 5HT synthesis were injected with vehicle alone or containing endotoxin (LPS) and killed 2 h later. The results indicate that the inhibition of the 5HT pathway did not modify the LPS-induced hypoglycemia but that it significantly reduced ACTH release in plasma. Although the inhibition of 5HT did not modify the CRH-ergic system, it diminished hypothalamic vasopressin (AVP) content in basal condition. After LPS injection, rats inhibited of 5HT synthesis showed a significant activation of the hypothalamic AVP-ergic system whereas control rats did not. These data clearly indicate that decreased HPA axis function after 5HT inhibition could be partially compensated by the facilitation of the AVP neuronal activity.

Key words: ACTH, glucocorticoid, CRH, vasopressin, serotonin, PCPA

Reciprocal interaction between the immune and neuroendocrine systems has been clearly established; for instance, cells from either system can express receptors for immune-derived cytokines, neurotransmitters and hormones; products from both systems coexist in cells from either system and mediators from one system can affect structures of the other one¹. These bidirectional interactions participate, as a consequence, in the organization of several neuroendocrine-immune circuits of pathophysiological significance for immunomodulation, host defenses and homeostasis². Endogenous glucocorticoids have been initially described as the link between the immune system and the hypothalamo-pituitary-adrenal (HPA) axis³. Regarding the most important interface between both systems, it has been demonstrated that the "in vivo" administration of immune-derived cytokines stimulate HPA axis activity⁴ by

acting mainly on the hypothalamus⁴ rather than at the anterior pituitary (AP) level⁴. It is well known that immune cells stimulated by microorganism-derived toxins, such as Gram-negative bacterial lipopolysaccharide (LPS), secrete cytokines⁵; in turn, these substances induce many host responses characterized by fever, stress hormone release, mineral redistribution, and increased acute phase protein synthesis⁶.

Many reports indicate that central monoaminergic mechanisms are involved in the regulation of the HPA axis function. Injection of serotonin (5HT) precursors, releasers, reuptake inhibitors, or receptor agonists of 5HT increase plasma ACTH and corticosterone⁷ levels in rodents. This evidence clearly indicates that central serotonergic neuronal system plays an important stimulatory role in the regulation of the HPA axis function; however, no significant data from the literature indicate a participation of this pathway in the endotoxin-stimulated HPA axis activity.

The aim of this study was to determine whether the central serotonergic pathway plays any role

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in HPA axis function during acute endotoxaemia. For this purpose, adult male Fischer rats (230 ± 30 g body weight) were used in these experiments; they were kept in standard conditions (07:00-19:00 lights on) and temperature (20 ± 2°C), with free access to food and water. All drugs were purchased from SIGMA, Chem. Co., unless indicated. Different groups (n = 8-10 rats per group; mean of three different experiments with 4-5 rats per group per experiment) were treated i.p. with 0.5 ml of saline solution alone (control group) or containing 50 mg/kg body weight of p-chlorophenylalanine (PCPA). PCPA inhibits irreversibly tryptophan-hydroxylase activity and induces a maximum 5HT depletion in brain at 24 h after treatment, which persists for at least one week⁸. At experimental time zero (26 hs after the first treatment) vehicle- and PCPA-treated rats were i.p. injected with vehicle either alone or containing LPS (L-3755; 130 µg/kg body weight) and killed, by decapitation, at 2 h after injection. After decapitation, trunk blood was collected and plasma samples were frozen until determination of glucose (G, enzymatic assay), corticosterone (B)⁹ and ACTH¹⁰ levels. Immediately after, brain tissues were quickly removed and the median eminence (ME), the medial basal hypothalamus (MBH) and the anterior pituitary (AP) were dissected as already described⁹. Tissues were then transferred to plastic tubes containing a small volume of 0.1 N HCl and sonicated. Samples were centrifuged and supernatants frozen until assayed for ME and MBH CRH and arginine-vasopressin (AVP) concentrations⁹ as well as AP ACTH content¹⁰. Data were statistically analyzed by multifactorial ANOVA followed by Fisher's test for comparison of different mean values¹¹.

The results indicate that PCPA treatment did not modify basal plasma G, ACTH and B levels (Fig. 1). As seen in Fig. 1 (upper panel), LPS treatment was able to induce a significant (P < 0.05) hypoglycemia in animals, regardless of any previous treatment. Figure 1 (middle panel) shows also that 2 h after the induction of the endotoxic shock (LPS groups) plasma ACTH levels were significantly (P < 0.01) increased over the respective baseline and that there was a marked difference between values attained in animals pretreated with PCPA and those reached in rats not treated with PCPA, in fact; plasma ACTH levels were significantly (P < 0.05) higher in the LPS

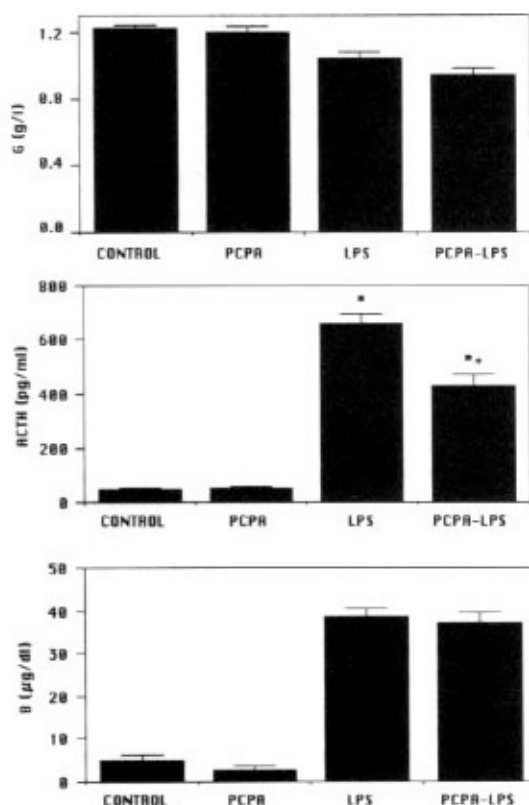


Fig. 1.— Plasma glucose (upper panel), ACTH (middle panel) and corticosterone (lower panel) levels at 2 h after injection of vehicle alone or containing LPS (130 µg/Kg body weight) in rats pretreated or not with PCPA. Values are the mean ± SEM (n = 8-10 rats per group; mean of three different experiments).

*, P < 0.05 or less vs. Control or PCPA values.

+, P < 0.05 vs LPS values.

than in the PCPA-LPS group of animals. Figure 1 (lower panel) shows also the results of plasma B levels in different groups of rats: LPS treatment induced a significant (P < 0.01) enhancement in plasma B levels over the respective basal values; however, no significant differences in plasma glucocorticoid concentrations were found between LPS-administered rats pretreated or not with PCPA, thus indicating that a maximal adrenal response was attained in both groups.

Table 1 shows the results of CRH and AVP contents in the ME and in the MBH as well as the AP ACTH content in the four experimental groups. ME CRH was slightly, albeit not significantly, decreased versus control values after either PCPA or LPS treatment. MBH CRH did not vary after all treatments. Interestingly, PCPA administration

TABLE 1.— Median eminence (ME) and hypothalamic (MBH) CRH (ng per specimen) and AVP (ng per specimen) contents as well as anterior pituitary (AP) ACTH concentration (ug per specimen) in control and PCPA-treated rats killed at 2 h after injection of vehicle either alone or containing LPS (130 ug/Kg; i.p.). Values are the mean \pm SEM ($n = 8-10$ rats per group; mean of three different experiments).

Group	Tissue	Peptide		
		CRH	AVP	ACTH
Control	ME	11.2 \pm 1.5	28.2 \pm 2.9	
	MBH	0.73 \pm 0.03	14.9 \pm 2.6	
	AP			0.97 \pm 0.11
PCPA	ME	9.0 \pm 1.0	18.9 \pm 2.3 ^a	
	MBH	0.66 \pm 0.08	7.2 \pm 2.1 ^b	
	AP			1.11 \pm 0.14
LPS	ME	9.3 \pm 1.3	24.7 \pm 2.8	
	MBH	0.86 \pm 1.02	17.6 \pm 3.0	
	AP			0.92 \pm 0.12
PCPA-LPS	ME	7.2 \pm 0.8	15.5 \pm 1.5 ^c	
	MBH	0.76 \pm 0.07	14.0 \pm 1.2 ^d	
	AP			1.17 \pm 0.15

^a, $P < 0.05$ vs. ME AVP values in Control

^b, $P < 0.05$ vs. MBH AVP values in Control

^c, $P < 0.05$ vs. ME AVP values in LPS.

^d, $P < 0.05$ vs. MBH AVP values in PCPA.

significantly ($P < 0.05$) decreased AVP content in both ME and MBH below the respective control values. While LPS treatment did not vary AVP content in both brain areas when compared with the respective control values, the immune challenge significantly ($p < 0.05$) increased ME AVP over the respective PCPA values, thus restoring such values to those found in control and LPS groups. Finally, none of the treatments modified AP ACTH.

The present results clearly indicate a role of the central serotonergic pathway on HPA axis function under endotoxemia. The modulatory effect of such a monoaminergic system appears to be of stimulatory nature. Although the participation of central serotonergic mechanisms in HPA axis activity has been previously reported^{7, 8}, our findings extend this observation to circumstances where an immune challenge (e.g., LPS) acts as stressor. It has been previously reported¹² that L-5-hydroxytryptophan, an aromatic L-aminoacid precursor of 5HT synthesis, "in vivo" administration increases HPA axis activity and that such a

mechanism, mainly mediated by 5HT1 receptors, is due to an enhancement of the hypothalamic CRH-ergic function. In the present study we have demonstrated that the hypoglycemic effect of endotoxin administration is preserved after 5HT synthesis inhibition, thus indicating a clear dissociation between different central monoamines activated during the endotoxemic shock. However, the LPS-elicited ACTH secretion was significantly lower in PCPA-treated rats than in controls; although a shift in time of the corticotrope response to LPS stimulation in PCPA-treated rats should not be ruled out, our results support a clear participation of the AVP-ergic system when the monoamine storage was decreased, in fact, PCPA treatment decreased both ME and MBH AVP but not CRH contents, findings independent of neuroimmune signals. However, this treatment also evidenced that the whole vasopressinergic (ME-MBH) system was activated by immune challenge administration (LPS), since a redistribution of AVP in the ME-MBH unit has taken place in

PCPA-treated animals under shock. In fact, after LPS injection, a slight decrease in MBH AVP together with a significant increase in ME AVP clearly indicate the reactivity of this neuronal system to immune challenge in PCPA-treated animals; whereas the administration of LPS in control rats did not change hypothalamic AVP neuron function. This observation could be indicating that 5HT modulates positively the hypothalamic vasopressinergic system; the lack of a normal 5HT-ergic tone not only decreases AVP-ergic function but also discloses a possible effect of released cytokines, during the acute phase of endotoxic shock, which are now able to stimulate the AVP neuronal pathway. It is known that AVP regulates AP ACTH output directly, potentiating CRH-induced ACTH release¹³ through AP V1b receptors¹⁴ and that AVP interacts with CRH and other neuropeptides of parvicellular origin¹⁵ released into the portal blood, so that it contributes to AP V1b receptor up-regulation. Therefore, it may be speculated that in conditions of diminished vasopressinergic function, such as after PCPA treatment, a development of more sensitive AVP neurons to released cytokines could play a partial compensatory role for maintaining a normal adrenal glucocorticoid secretion thus contributing to the normal host's defense mechanisms following injury.

Resumen

Participación de la vía central serotoninérgica en la activación del eje hipotálamo-hipófiso-adrenal durante el shock endotóxico

El presente trabajo tuvo como objetivo el determinar si existe una participación de la vía central serotoninérgica (5HT) sobre la activación del eje hipotálamo-hipófiso-adrenal (HPA) durante el shock endotóxico (LPS). Nuestros experimentos indicaron que la inhibición de la síntesis de 5HT no modificó la hipoglucemia ocurrida a las 2 h posteriores a LPS aunque sí disminuyó la liberación de ACTH en plasma. La falta de 5HT, si bien no modificó la actividad neuronal CRH, disminuyó la cantidad de vasopresina (AVP) hipotalámica basal. La inyección de LPS, en ratas pretratadas con un inhibidor de la síntesis de 5HT, reveló que el sistema neuronal de AVP resultó activado, mientras que el tratamiento con LPS no reprodujo dichos efectos en las ratas controles. Nuestros resultados sugieren que la disminución de la activi-

dad del eje HPA durante el shock endotóxico, como consecuencia de la inhibición de la síntesis de 5HT, estaría parcialmente compensada por una facilitación de las neuronas hipotalámicas productoras de AVP.

References

1. Besedovsky HO, Del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. *Endocr Rev* 1996; 17: 64-102.
2. Munck A, Guyre PM, Holbrook, NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev* 1984; 5: 25-44.
3. Fauci AS. Immunosuppressive and anti-inflammatory effects of glucocorticoids. In: Baxter JD, Rosseau GG, (eds). Glucocorticoid hormone action. New York: Springer, 1979; 449.
4. Sharp BM, Matta SG, Peterson PK, et al. Tumor necrosis factor is a potent ACTH secretagogue: comparison to interleukin-1. *Endocrinology* 1989; 124: 3131-3.
5. Dinarello CA, Mier JW. Lymphokines. *N Engl J Med* 1987; 317: 940-5.
6. Dinarello CA. The biology of interleukin-1. *FASEB J* 1988; 2: 108-15.
7. Fuller RW. The involvement of serotonin in regulation of pituitary-adrenocortical function. *Front Neuroendocrinol* 1992; 13: 250-70.
8. Fuller RW. Serotonergic stimulation of pituitary-adrenocortical function in rats. *Neuroendocrinology* 1981; 32: 118-27.
9. Spinedi E, Giacomini M, Jacquier MC, Gaillard RC. Changes in the hypothalamo-pituitary-adrenal axis after bilateral adrenalectomy: evidence for a median eminence site of glucocorticoid action. *Neuroendocrinology* 1991; 53: 160-70.
10. Hodgkinson SC, Allolio B, Landon J, Lowry PJ. Development of a non-extracted two-site immunoradiometric assay for corticotropin utilizing extreme amino- and carboxy-terminally directed antibodies. *Biochem J* 1984; 218: 703-11.
11. Zar JH. Biostatistical Analysis. Englewood Cliffs: Prentice-Hall, New Jersey, 1974.
12. Spinedi E, Gaillard RC. Stimulation of the hypothalamo-pituitary-adrenocortical axis by the central serotonergic pathway; involvement of endogenous corticotropin-releasing hormone but no vasopressin. *J Endocrinol Invest* 1991; 14: 551-7.
13. Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol* 1994; 15: 321-50.
14. Jard S, Gaillard RC, Gullon G, et al. Vasopressin antagonists allow demonstration of a novel type of vasopressin receptor in the rat adenohypophysis. *Mol Pharmacol* 1986; 30: 171-7.
15. Sawchenko PE, Imaki T, Potter E, et al. The functional anatomy of corticotropin releasing factor; Corticotropin Releasing Factor. In: Chadwick DJ, Marsh J, Akkrill K, (eds). Ciba Foundation Symp 172. New York; Wiley and Sons, 1989; 5-26.