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MECHANISMS IN THE CONTROL OF STRESS RESPONSIVENESS

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Summary. - *Evidence is given of cooperation between pituitary desensitization to the stimulatory action of corticotropin-releasing hormone (CRH) and glucocorticoid negative feedback in the modulation of the stress responsiveness. With regard to the former, we show that the pituitary becomes unresponsive to repeated CRH administration as soon as 15 min after the first one, while the adrenocortical effect of arginine-vasopressin (AVP) during this period is amplified, suggesting the involvement of AVP in the mechanism that permits repeated pituitary-adrenocortical axis activations. The activation of this axis is blocked by the glucocorticoid negative feedback induced by a previous stress. In fact, after a cold stress (4-6 °C for 90 min), the responsiveness to a subsequent psychic stressor (but not to a somatic one) is suppressed. Results after neurotoxic lesion of hippocampal and hypothalamic serotoninergic innervations, would indicate that the neurotransmitter is in some way involved in the accomplishment of this phenomenon.*

Riassunto (Meccanismi nel controllo della responsività allo stress). - *Viene data la prova della cooperazione tra la desensibilizzazione ipofisaria all'azione stimolatoria dell'ormone rilasciante la corticotropina (CRH) ed il* feedback *negativo glucocorticoideo nella modulazione della responsività allo stress. Per quanto riguarda la desensibilizzazione, mostriamo che l'ipofisi diventa non responsiva a ripetute somministrazioni di CRH già 15 min dopo la prima somministrazione, mentre l'effetto adrenocorticale della arginina-vasopressina (AVP) durante questo periodo è amplificato, suggerendo il coinvolgimento della AVP nel meccanismo che permette ripetute attivazioni dell'asse ipofisi-corticosurrene. L'attivazione di questo asse è bloccata dal* feedback *negativo glucocorticoideo indotto da un precedente stress. Infatti, dopo uno stress da freddo (4-6 °C per 90 min), la responsività ad un seguente stressor di natura psichica (ma non di natura somatica) è soppressa. Risultati dopo lesione neurotossica delle innervazioni serotoninergiche ippocampali e ipotalamiche indicherebbero che il neurotrasmettitore è in qualche maniera coinvolto nell'attuazione di questo fenomeno.*

Mechanisms in the control of stress responsiveness

Although much is known on the nervous and neuroendocrine mechanisms of initiating a pituitary-adrenocortical response to a psychic stressor, relatively scanty is the knowledge of how these mechanisms operate in the case of sequential stresses at intervals of minutes or hours. In this evenience the psychic stressors can be the same, or differ so that habituation cannot have place. With regard to these mechanisms, it has been demonstrated that desensitization to corticotropin releasing hormone (CRH) occurs both *in vitro*, with a down regulation of the relevant receptors [1] and *in vivo* [2, 3], so that factors other than CRH must concur to maintain the responsiveness to sequential psychic stressors. On the other hand, it is to be expected that this responsiveness should be modulated by a negative feedback operated by the glucocorticoid hormone, secreted in the previous stress, on the response to a subsequent stressor. However, this possibility has been advocated [4, 5] or demonstrated [6, 7] in few circumstances, or even excluded of any actuality [8], possibly because of its being counteracted by a prolonged period of hypersensitiveness in either the central nervous system or anterior pituitary components of the adrenocortical system, left over by the previous stress [9].

In the present contribution to the study of how repeated stress activation of the pituitary-adrenocortical axis is modulated, we report an instance in which the pituitary maintain its responsiveness to short interval repeated stressors, in spite of the fact that repeated administration of CRH with the same cadence induces a condition of pituitary refractoriness to the releasing signal. As shown in Table 1, the

Table 1. - *The pituitary-adrenocortical response to repeated 2 min-restraint in the rat, and the sensitiveness of pituitary to CRH (100 ng/rat) or AVP (100 ng/rat) intravenous administration in chlorpromazine-morphine-nembutal hypothalamus blocked rats*

Treatment		Plasma corticosterone µg/100 ml
0 min	15 min	30 min
nothing	nothing	1.38 ± 0.19 (6)
restraint	nothing	13.21 ± 3.20 (6)
restraint	restraint	53.87 ± 14.89 (*a*) (5)
vehicle	vehicle	0.64 ± 0.36 (4)
CRH	vehicle	23.01 ± 5.11 (7)
CRH	CRH	27.65 ± 3.73 (6)
vehicle	AVP	1.20 ± 0.45 (4)
CRH	AVP	46.50 ± 4.52 (*b*) (6)

(*a*) and (*b*) 0.01 per cent significant difference versus restraint/nothing, or versus CRH/CRH; Duncan's New Multiple Range test.

Table 2. - *The temporal course of the negative feedback exerted by a previous stress on the responsiveness to a subsequent psychic stressor (psychic stress -feedback) in the rat*

	Plasma corticosterone µg/100 ml	Plasma ACTH pg/ml
Basal	1.10 ± 0.14 (23)	36.50 ± 3.50 (6)
Psychic stress (*a*)		
at 5 min		65.00 ± 5.10 (*g*) (7)
at 15 min	6.99 ± 0.41 (20)	52.00 ± 3.70 (7)
Cold stress (*b*)		
at 90 min	21.99 ± 1.83 (15)	
Cold stress:		
45 min after	0.94 ± 0.05 (15)	
Psychic stressor (*c*)		
45 min after		
Cold stress:		
at 5 min		44.00 ± 2.50 (*h*) (8)
at 15 min	1.75 ± 0.24 (24) (*e*)	44.00 ± 3.40 (7)
Ether stressor (*d*)		
45 min after		
Cold stress:		
at 15 min	26.30 ± 3.04 (5)	
Psychic stressor		
180 min after		
Cold stress:		
at 15 min	7.70 ± 1.87 (6) (*f*)	
Ether stressor		
180 min after		
Cold stress:		
at 15 min	23.17 ± 2.92 (6)	

(*a*): change of cage and room for 1 min; (*b*) 90 min at 6 °C; (*c*): as in a; (*d*): 1 min exposure to vapor. ANOVA and Mann Whitney "U" test; (*e*): 1 percent significant difference *vs* psychic stress 90 min at 6 °C; (*f*): percent significant difference *vs* psychic stressor 45 min after cold stress. ANOVA and Duncan's New Multiple Range test; (*g*): 1 percent significant difference *vs* basal; (*h*): 1 percent significant difference *vs* psychic stress at 5 min.

application of a second restraint as a stressor 15 min after a first one is able to fully produce an adrenocortical response. However, the administration of CRH is unable in the chlorpromazine-morphine-nembutal hypothalamic blocked rat to activate the pituitary-adrenocortical secretion 15 min after a first dose, chosen from a dose-response curve as that producing maximal activation. At this moment, a dose of arginine-vasopressin (AVR) chosen from a dose-response curve as a subeffective one on its own account, is able to activate the pituitary-adrenocortical secretion 15 min after CRH administration. Considering that the stress procedure used in this experiment (2 min in a plexiglass chamber) has a psychic component prevalent over the somatic one, it can be envisaged that responsiveness of the pituitary-adrenocortical axis to short interval repeated psychic stress procedures may be maintained through the conjunct action of CRH and AVP. In the lack of a reliable anti-AVP antiserum, we could not obtain the effective demonstration of this evenience. At any rate, our findings are in agreement with the demonstration of the complex role played by the interaction between CRH and AVP on ACTH release *in vivo* [10, 11].

Opposite to the above, there is an instance in which a "stress-feedback" is evident in the rat, namely a negative feedback operated by a previous stress, suppressing the hypotalamo-pituitary-adrenocortical responsiveness to a subsequent psychic stressor. This is shown in Table 2: 45 min after a cold stress (at 4-6 °C for 90 min), a pure psychic stress procedure (change of cage and room for 1 min) is unable to elicit an adrenocortical response. However, if ether vapor is instead applied as a stressor, after the same interval, a full adrenocortical response is obtained. To be noted that at the time of exposure to the second stressor, the plasma corticosterone concentration has regained basal values. In Table 2 it is also shown that the failure to produce a second response is not due to insensitiveness of the adrenal cortex to adrenocorticotropin (ACTH) stimulation (the concentration quotient plasma corticosterone/ plasma ACTH remains unchanged), but to an absence of pituitary activation. The psychic stress-feedback appeared as a highly reproducible consistent phenomenon: in 106 control rats (not prestressed) the plasma adrenocortical response to the pure psychic stressor was 14.16 ± 0.8 from a basal value of 2.07 ± 0.23 µg/100 ml, whereas was 5.07 ± 0.53 from a post-stress recovered value of 2.34 ± 0.34 µg/100 ml in 113 prestressed rats, with a statistically significant difference at a 1 percent level (non parametric ANOVA test). It is surprising how this regulatory phenomenon of the psychic stress responsiveness could have been passed unnoticed by the authors who have looked into the physiology of the glucocorticoid negative feedback at the level of the pituitary and/or hypothalamus. It is to be emphasized that this regulatory mechanism appears

specific for psychic stress, in which activation of the hypothalamus CRH releasing activity is operated by suprahypothalamic brain structures, while activation of the hypothalamus by ether can be obtained even in the presence of its total deafferentation [12].

A number of works point to an inhibitory role of the hippocampus on stress responsiveness of the hypothalamo-pituitary-adrenocortical axis [13-16] possibly mediated by the adrenocorticoid receptors in this structure (for a review, see [17]), the binding capacity of which is regulated by the serotoninergic innervation [18]. Moreover, a relationship has been demonstrated between serotonin turnover in the hippocampus and the level of adrenocortical secretion [19]. On this basis we have investigated the role of serotoninergic innervation in the production of the psychic stress-feedback.

As shown in Table 3, 8 days after the neurotoxic lesion of the serotoninergic system at the dorsal raphe level in the rat, basal and stress plasma corticosterone levels are the same as in blank and sham lesioned animals. However, the psychic stress-feedback is substantially suppressed. At this time, as shown in the same Table, serotonin and 5-hydroxy-indolacetic acid concentrations are strongly reduced in both hippocampus and hypothalamus: 61 and 66, and 65 and 67 percent, respectively, so that no distinction between the two structures was possible with regard to their possible role in the production of the psychic-stress feedback. A more selective neurotoxic lesion of the serotoninergic innervation to the hippocampus, produced at the level of the fimbria-formix plus cingulum bundle, was unable, as shown in Table 3, to suppress the psychic stress-feedback in presence of a 67 and 34 percent reduction in the concentration of serotonin and 5-hydroxy-indolacetic acid in the hippocampus, respectively. A reduction of a similar degree (53 percent) in the serotonin concentration, not accompanied by a reduction in 5-hydroxy-indolacetic acid concentration, was present in the hypothalamus, for which, considering the anatomical location of the neurotoxic lesion, no explanation is readily available, unless a strong compensatory reduction of serotoninergic activity in this structure is thought of. To be noted that 21 days after the neurotoxic lesion at the level of the dorsal raphe, the psychic stress-feedback was regained, although an important reduction of the neurotrasmitter persisted in both hippocampus and hypothalamus (not shown). Because the increase in plasma corticosterone during stress produces occupation of the adrenocorticoid receptor in the hippocampus [20], specifically of the glucocorticoid preferring type, type II [21], followed by a transitory reduction in cytosolic binding capacity in this structure (down regulation [22, 20]), it would appear that the glucocorticoid signal and changes in brain serotoninergic activity concur in determining an adjustment of the sensitiveness to repeated psychic stress in the rat.

The ability of the rat's brain to maintain its neuroendocrine responsiveness to shortly subsequent psychic stressor, and, on the opposite, to dampen it to repeated presentation of stressful situations at relatively longer intervals, reveals the existence of an order in the regulation of stress responsiveness which can be disturbed if there is an improper function of the serotoninergic system. This notion can be intriguing when considering that in endogenus depressive patients, in the majority of whom a disorder in the regulation of the HPAA is present, such dysfunction has been postulated, and that in this condition serotoninergic drugs are largely resorted to.

Table 3. - *The psychic stress-feedback (psychic stressor 45 min after a 90 min cold stress) in rats 8 days after a 5,7-dihydroxy-tryptamine lesion in two areas of the brain serotoninergic system*

Dorsal Raphe

Plasma corticosterone: µg/100 ml

	Basal	Psychic stress	Psychic stress feedback
Lesioned (*a*)	1.29 ± 0.13 (10)	7.95 ± 0.81 (20)	6.81 ± 1.02 (18) (*c*)
Sham + blank (*b*)	2.40 ± 0.50 (5)	9.73 ± 1.35 (8)	3.65 ± 0.62 (8) (*d*)

Serotoninergic system: residual percent in lesioned *vs* sham + blank (*e*)

	5-OH tryptamine	5-OH indolacetic
Hippocampus (9)	39.07 ± 4.21	34.34 ± 5.97
Hypothalamus (9)	34.59 ± 3.66	33.02 ± 4.75

Fimbria fornix + cingulum bundle

Plasma corticosterone: µg/100 ml

	Basal	Psychic stress	Psychic stress feedback
Lesioned	3.32 ± 1.30 (7)	22.09 ± 2.91 (7)	10.55 ± 2.61 (9) (*f*)
Sham	2.77 ± 1.04 (8)	29.20 ± 2.74 (9)	10.31 ± 2.85 (9) (*f*)

Serotoninergic system: residual percent in lesioned *vs* sham + blank (*e*)

	5-OH tryptamine	5-OH indolacetic
Hippocampus (14)	32.57 ± 2.26	47.05 ± 4.67
Hypothalamus (15)	65.98 ± 3.25	83.30 ± 4.23

(*a*): considered as such when concentration of 5-hydroxy-tryptamine was lower than the mean minus two standard deviations in the sham animals; (*b*): no difference was found between the two groups; (*c*): no statistically significant difference *vs* psychic stress; (*d*): 1 percent difference *vs* psychic stress; (*e*): ≥ 1 percent significant difference; (*f*): 1 percent significant difference *vs* psychic stress; Duncan's New Multiple Range test.

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