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Interaction of Tonic Immobility and Dexamethasone in the Modulation of Hippocampal 5-HT Activity in Rabbits

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FARABOLLINI, F., L. LODI AND C. LUPO. *Interaction of tonic immobility and dexamethasone in the modulation of hippocampal 5-HT activity in rabbits.* PHARMACOL BIOCHEM BEHAV **25**(4) 781–784, 1986.—The immobility reaction (animal hypnosis) in the rabbit is related to basal levels of corticosterone and to modifications in 5-HT activity in the brain. In order to investigate the relationship between adrenocortical function, brain 5-HT activity and immobility response in rabbits, the susceptibility to the reaction and 5-HT and 5-HIAA levels in the hippocampus were studied in animals whose pituitary-adrenal function was altered by chronic DEX treatment. It was still possible to induce immobility in animals treated with DEX. The reduction in duration observed under the present testing schedule (two sessions of immobility separated by a one week interval) did not occur in the treated animals. There was a reduction in 5-HT turnover as a consequence of the immobility response and this confirms previous findings in other brain areas of the same species. In animals pretreated with DEX, so as to drastically reduce ACTH and corticosterone, not only was a reduction in 5-HT turnover prevented, but there was also an increase in 5-HT and 5-HIAA levels when immobility was induced. These results are discussed in terms of the influence of the pituitary-adrenal system in the neuroendocrine and behavioural aspects of the immobility reaction.

Immobility reaction (animal hypnosis) Corticosteroids Dexamethasone Serotonin (5-HT) 5-Hydroxy-indolacetic acid (5-HIAA)

ANIMAL hypnosis, a condition of transitory tonic immobility which can be induced by physical restraint, has been regarded as a form of adaptive behavior in the presence of threatening stimuli. The duration of immobility varies according to species and subject. In a given animal it is largely dependent upon the type of testing schedule used, by means of which either habituation [6] or sensitization [5] may be obtained.

Previous work in the rabbit indicates that susceptibility to the immobility reaction is correlated with corticosterone plasma levels [2] and its suppression through habituation is associated with an increase in circulating corticosterone [6], thus suggesting the involvement of the pituitary-adrenocortical axis.

Serotonin has been shown to be the main mediator for the immobility response in two different types of experiments. On the one hand the pharmacological manipulation of the serotonergic system is reflected in modifications in duration, usually with an inverse relationship between serotonergic activity and duration of immobility [1, 7–9, 17]. On the other, the elicitation of immobility is associated in the rabbit with reduced levels of 5-HT in brain areas such as the pons-medulla, mesencephalon and striatum [5].

There is experimental evidence of a close correlation between brain serotonin (5-HT) and pituitary adrenal function: decreased turnover of 5-HT was observed in the hippocampus following adrenalectomy [3] and this effect was counteracted by treatment with either corticosterone or DEX [16]. It is not unlikely that the behavioral changes effected by corticosteroid manipulation be related to changes in serotonergic activity in the brain.

It is also known that the hippocampus is an important component of the limbic structures implicated in the control of adaptive behavior [12]. Protein binding sites for adrenal corticosteroids are mainly concentrated in this area. The synthetic glucocorticoid DEX, known to inhibit the corticotrophic stimulation of the adrenal cortex, binds to these receptor sites though with less affinity.

In the present experiments, the alteration of pituitary-adrenal function by DEX was investigated by examining the changes in hippocampal 5-HT and 5-hydroxy-indolacetic acid (5-HIAA) levels along with the concomitant effects on susceptibility to animal hypnosis.

METHOD

Subjects

New Zealand male adult rabbits (Orictolagus Cuniculus) of 2700 g body mean weight were used in this study. They were individually housed and had free access to food and water.

Procedure

The animals were divided into five groups of seven animals: Group 1—Control (untreated) animals; Group

TABLE 1

EFFECT OF DEXAMETHASONE ON IMMOBILITY DURATION IN RABBIT

Group		Mean of Four Trials	Significance*
Immobility (Gr. 3)	First session	108 ± 103	$p<0.05$
	Second session	65 ± 56	
Immobility+DEX (Gr. 5)	First session	75 ± 121	n.s.
	Second session	113 ± 194	

Means (sec) and SD
*Wilcoxon test (two tailed).

TABLE 2

EFFECT OF IMMOBILITY WITH OR WITHOUT DEXAMETHASONE TREATMENT ON ACTH AND CORTICOSTERONE PLASMA LEVELS IN MALE RABBITS

	ACTH (pg/ml)		Corticosterone (μg/100 μl)	
	Sample 1st	Sample 2nd	Sample 1st	Sample 2nd
(1) Control	117 ± 38	134 ± 31	7.3 ± 3.3	8.9 ± 5.2
(2) Induction	108 ± 33	397 ± 67†	26.9 ± 15.4	88.8 ± 16.4*
(3) Immobility	113 ± 24	536 ± 77‡	11.3 ± 9.8	56.4 ± 13.1†
(4) Dexamethasone	51 ± 16	0 ± 0†	4.8 ± 4.2	0.4 ± 0.3*
(5) Immobility + DEX	206 ± 32	3 ± 1‡	7.3 ± 2.6	0.11 ± 0.01†

Means and SE.
Paired t test: *$p<0.05$; †$p<0.02$–0.01; ‡$p<0.005$–0.001

2—Animals undergoing induction alone in two sessions separated by a one week interval; Group 3—Animals immobilized in two sessions separated by a one week interval; Group 4—Animals injected daily with 0.2 mg/kg of DEX (Decadron) for seven days; Group 5—Animals immobilized as in group 3 and treated with DEX as in group 4 in the intervening week.

Immobility was induced by inversion and restraint of the rabbit in a wooden trough [10]. The duration of the induction procedure never exceeded 30 sec; induction was not repeated when it failed to induce immobility. The immobility episode was considered to begin with the disappearance of righting reflexes, and to end with the first movement. Immobility episodes were never purposely interrupted by the experimenter. In group 2 the animals underwent the procedure of induction by inversion, but the immobility response was not permitted to occur [4]. In each session animals received four series of immobility trials or induction alone with no intertrial interval.

Biochemical Assays

Blood samples were collected twice; the first at the beginning of treatment and the second when the animals were sacrificed, the day after the last DEX injection (group 4) and 15 min after the last episode of immobility or induction (groups 2, 3 and 5). In the control animals (group 1) blood samples were also taken with a one week interval. 5-HT and 5-HIAA were measured by fluorimetry according to the method of Maickel *et al.* [11] modified by Miller *et al.* [14].

ACTH levels were determined by radioimmunoassay performed directly in the plasma samples (0.1 ml), as follows. ^{125}I-ACTH and anti-ACTH antiserum were added to the plasma; the mixture was left at room temperature for 48 hours and then the free hormone was separated from the bound by the addition of a charcoal suspension. After centrifugation the radioactivity of the supernatant was determined by gamma count. Corticosterone levels were determined by radioimmunoassay after extraction of the plasma with methylene chloride. Tritiated cortisol and antiserum anti-corticosterone were added in buffer phosphate to the dry evaporated extract, the mixture incubated for 3 hours at 4–8°C, the free steroid separated from the bound by addition of a charcoal-dextran mixture (0.25% Norit A, 0.025% dextran T 70, 0.5 ml). After centrifugation the radioactivity was determined in the supernatant by liquid scintillation count.

RESULTS

Table 1 shows the mean duration ± SD of the four trials of each session in the groups of animals which were immobilized. In group 3 a significant decrease in the duration of immobility was observed between the first and the second session. In group 5 (immobility and DEX treatment) there

TABLE 3

EFFECT OF IMMOBILITY WITH OR WITHOUT DEXAMETHASONE TREATMENT ON 5-HT AND 5-HIAA LEVELS IN THE HIPPOCAMPUS OF MALE RABBITS

	(1) Control	(2) Induction	(3) Immobility	(4) Dexamethasone	(5) Immobility + DEX
5-HT	0.54 ± 0.19	1.17 ± 0.62	0.39 ± 0.11	0.60 ± 0.39	1.07 ± 0.29
5-HIAA	0.87 ± 0.38	0.84 ± 0.24	0.32 ± 0.18	0.43 ± 0.12	0.81 ± 0.29
5-HIAA/5-HT	1.58 ± 0.34	0.86 ± 0.50	0.82 ± 0.50	1.08 ± 0.92	0.76 ± 0.22

Means and SD, LSD test.
*$p<0.05$; †$p<0.02$–0.01; ‡$p<0.005$–0.001.

was not such decrement but a tendency (albeit not significant) to increase. The Mann-Whitney test applied to the first sessions of the two groups was not significant, showing that the two groups were initially homogeneous (U=46; n.s.).

Table 2 shows plasma levels of ACTH and corticosterone before and after different treatments. A significant increase in these hormones was observed after either induction or immobility. Treatment with DEX alone or in combination with immobility resulted in a drastic reduction in ACTH and corticosterone. Variance analysis of the first samples from the five groups was not significant either for corticosterone, $F(4,30)=1.35$, n.s., or for ACTH, $F(4,30)=2.91$, n.s., showing that the animals of the groups were drawn from a homogenous population.

Variance analysis of hippocampal 5-HT and 5-HIAA data showed significant differences between groups for 5-HT, $F(4,30)=7.84$, $p<0.001$, 5-HIAA, $F(4,30)=5.33$, $p<0.002$, and the 5-HIAA/5-HT ratio, $F(4,30)=3.51$, $p<0.02$. Group comparison was performed by the Least Significant Difference (LSD) test [15]. Means, standard deviations and levels of significance are reported in Table 3. Values of 5-HT in groups of animals which were immobilized or treated with DEX did not differ from control values but when the two treatments were combined (group 5) a significant increase was observed with respect to the control group (group 1) and the immobility (group 3) and DEX (group 4) groups. In the group of animals which only underwent the procedure of induction, 5-HT values were higher than in the immobility group (group 3). Other differences were not significant.

5-HIAA levels were lower in the immobility and DEX groups (groups 3 and 4) than in controls but not in the combined treatment group (group 5) which showed significantly higher values than those observed in the separate treatment groups (groups 3 and 4). Moreover the immobility group displayed lower 5-HIAA levels than the induction group, which was very similar to controls. The 5-HIAA/5-HT ratio in the induction (group 2), immobility (group 3) and immobility+DEX (group 5) groups was lower than in the controls, whereas it was unmodified in the DEX group (group 4).

DISCUSSION

Behavioral data obtained in the present experiments show that the duration of the immobility reaction is decreased when animals are tested in two sessions separated by a one week interval. When animals are treated with DEX, in a dose that drastically reduces ACTH and corticosterone, in the interval between the two sessions, immobility can still be induced. Furthermore, not only is there no decrement in duration in the second session, but, if anything, there is a slight increase.

These findings indicate either that the integrity of the pituitary-adrenal system is not necessary for the elicitation of the immobility reaction, or that DEX itself is able to substitute for the natural corticosteroids in this respect. The second possibility seems more likely, due to a correlation previously found between corticosterone plasma levels and susceptibility to the immobility reaction [2].

If a decrement in duration is regarded as a step in the process of habituation [6], the present findings may be an indication of the involvement of pituitary-adrenal system in the development of habituation. This possibility might be verified in experiments properly planned for the study of habituation.

The biochemical data here obtained show that serotonin metabolism is differentially affected by immobility depending on whether the pituitary-adrenal system is intact or suppressed. In the present experiments immobility treatment led to a reduction in 5-HT turnover. This is in line with previous observations in other brain areas of the same species, in experiments carried out with different schedules of immobility elicitation [5]. Our results also confirm that the effect on 5-HT turnover is not due to the manipulative procedure of induction, since the latter increases 5-HT synthesis without modifying catabolism.

The effect of the combined immobility+DEX treatment is of particular interest. Pretreatment with the synthetic glucocorticoid not only prevents the reduction in 5-HT turnover consequent to immobility alone, but also increases

5-HT and 5-HIAA levels. This finding indicates that, unlike the behavioral aspect of immobility, the effect of the immobility itself on the serotonergic system requires the integrity of the pituitary-adrenal system.

The effect obtained with the combined treatment is also different from that obtained with DEX alone the latter consisting in a reduction in 5-HIAA production. This finding is in line with the decreased brain turnover of 5-HT found in rats as a consequence of reduced corticosterone levels after adrenalectomy [16].

It is known that adrenal glucocorticoids are selectively concentrated in neurons of the hippocampus; however a different pattern has been recognized for the synthetic glucocorticoid DEX than for corticosterone [4]. In some experiments DEX is reported to be able to mimic the effects of the natural glucocorticoids, whereas in others its effects are different from those of corticosterone [12].

A likely explanation of our data is that DEX, although able to substitute the natural glucocorticoids in controlling the duration of the immobility response, is not equally effective on the serotonergic system. It also seems that the suppression of ACTH release by DEX administration is not crucial to the immobility response but no conclusion can be drawn as to whether the effect of DEX on 5-HT is exerted at the hippocampal receptor level or on ACTH release.

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