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Neurotrophic peptide ACTH-(4-10) permits glucocorticoid-facilitated retention of acquired immobility response of hypophysectomized rats

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The Porsolt swimming test, which was originally designed as an experimental model to screen potential antidepressants demands that rats be forced to swim for 15 min in a narrow cylinder. Twenty four hours later they are retested for 5 min during which they stay immobile for approximately 70% of the time. The present study showed that hypophysectomized animals, 14 days after operation, were unable to retain the acquired immobility. Subcutaneous glucocorticoid administration did not restore the retention of acquired immobility in contrast to our earlier finding with adrenalectomized animals. The deficit in responsiveness to glucocorticoids was eliminated when the hypophysectomized rats received ACTH-(4-10)s.c. (20 μg/rat) every other day. Chronic treatment with only the peptide did not improve the impaired retention of hypophysectomized rats and a single ACTH-(4-10) injection 1 day or 1 h prior to initial testing was also ineffective. We conclude that the neurotrophic peptide ACTH-(4-10) permits the expression of the glucocorticoid effect on retention of acquired immobility in the swimming test.

ACTH-(4-10); Immobility, acquired; Glucocorticoid receptors; Hypophysectomy; Swimming behaviour

1. Introduction

In 1977 Porsolt and colleagues described a test to screen potential antidepressants. In this test the rats are forced to swim for 15 min in a narrow glass cylinder containing 15 cm of water (Porsolt et al., 1977). During these 15 min the rats become progressively more immobile. When retested for 5 min 24 h later the animals have retained this immobility response and stay immobile for approximately 70% of this period.

Bilateral adrenalectomized animals failed to retain the acquired immobility. This deficit could be repaired by administration of either κ-selective opioid peptides or glucocorticoids within one hour after the initial test (Jefferys et al., 1983; 1984; 1985; Veldhuis et al., 1985). It was postulated that a post-stress rise in these hormones facilitated the incorporation of the information after the initial test via a direct action on the brain (Jefferys et al., 1983).

Hypophysectomized animals are disturbed in the performance of several conditioned behaviours. ACTH-(4-10) replacement can correct these behavioural impairments (De Wied, 1969). The present study was designed to investigate the performance of hypophysectomized animals in the swimming test. We showed that hypophysectomized rats were deficient in retaining the acquired immobility and that neither ACTH-(4-10) nor dexamethasone restored the immobility response but that pretreatment with the neurotrophic peptide was a necessary requirement for the expression of the glucocorticoid effect.

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2. Materials and methods

2.1. Animals

Male Wistar albino rats from the Central Breeding Laboratory TNO (Zeist, The Netherlands) weighing 150-180 g at the beginning of the experiment were used. The animals were housed in groups of 5-6 per cage and received food and tap water ad libitum. Hypophysectomized animals received a 5% glucose solution as drinking solution. All animals were kept under a controlled light-dark schedule (light on between 06:00 and 20:00 h) and the temperature was maintained at 22° C.

2.2. Surgery

Hypophysectomy and sham operation was performed via the transauricular route under ether anaesthesia. Loss of body weight and macroscopic inspection of the sella turcica and adrenal atrophy served as criteria for the completeness of hypophysectomy. Our hypophysectomized animals lost up to 20% of their body weight within three days after surgery and their body weight thereafter stayed at this level. Only data from completely hypophysectomized animals are presented. The hypophysectomized animals were used in the experiments 14 days after operation.

2.3. Test procedure

The swimming test developed by Porsolt et al. was used (Porsolt et al., 1977; 1978). The rats were placed in narrow glass cylinders (height 25 cm, diameter 18 cm), containing 15 cm of water at 25° C for 15 min (initial test), followed by a 5 min retest 24 h later. Absence of hindleg movement, as a measure of immobility, was recorded with an electronic timer during the retest. After swimming the rats were dried and put in a heated room (37° C) for 30 min. All tests were performed between 8:30 and 12:30 a.m.

2.4. Experiments

For the first experiment hypophysectomized rats treated with ACTH-(4-10) (0.2 ml containing 20 μg ACTH-(4-10)) (De Wied, 1966) or with zinc phosphate vehicle and sham-operated rats treated with vehicle were tested two weeks after surgery. ACTH-(4-10) was given for 13 days as a subcutaneous injection (ACTH-(4-10) or vehicle) every other day, starting the day after operation. The last injection was given the day before the initial test. Some animals were used for obtaining the acquisition curve: immobility was measured in the initial test divided into three time intervals of 5 min. Fifteen minutes after the initial test the animals received 10 μg dexamethasone per 100 g b.w. and the control animals received vehicle only. Immobility of all animals was measured in the retest on the following day.

In the second experiment hypophysectomized animals received the same zinc phosphate preparation of ACTH-(4-10) as replacement therapy. Control animals were treated with the vehicle only. Three groups of ACTH-(4-10)-pretreated rats as well as three groups of vehicle-pretreated rats received a s.c. dexamethasone injection 15 min after the initial test (3-30 μg/100 g body weight); the control groups received vehicle only. Dexamethasone was dissolved in ethanol and was diluted with polyethylene glycol to a final concentration of 2% ethanol.

In the final experiment, both the hypophysectomized and the sham-operated animals treated with zinc phosphate vehicle until the initial test were tested two weeks after surgery. The rats received a single ACTH-(4-10) injection (20 μg in zinc phosphate vehicle 24 h or 1 h before the initial test. The hypophysectomized animals were treated with dexamethasone (10 μg/100 g b.w. s.c.) 15 min after the initial test. The control rats received vehicle only.

2.5. Statistics

An analysis of variance was applied to the data, followed by post hoc comparisons of group means by the Duncan's multiple range test (SPSS program). A P value of 0.05 or less was taken to indicate a significant difference.

3. Results

The effect of hypophysectomy on the acquisition of the immobility response and the retention

of that response is shown in fig. 1. Hypophysectomy did not influence the acquisition rate for the behaviour although the performance level of sham-operated animals was never reached. Hypophysectomy impaired the retention of acquired immobility when compared to sham-operated animals, at one week (percentage immobility, hypophysectomized animals: $34.3 \pm 4.5\%$ ($n = 22$); sham-operation-hypophysectomy: $66.9 \pm 6.5\%$ ($n = 7$)) and at two weeks after surgery (fig. 1).

Dexamethasone was unable to restore the immobility of hypophysectomized animals tested two weeks after operation to the level of the sham-operated animals (fig. 1). However, after chronic pretreatment with ACTH-(4-10) doses of 10 and 30 μg dexamethasone effectively improved the deficient immobility response when compared to vehicle-pretreated hypophysectomized animals. This improvement of the immobility response was dose-dependent (fig. 2). The retention of immobility of ACTH-(4-10) treated hypophysectomized animals after dexamethasone treatment was comparable to that of sham-operated animals but not to that of vehicle-treated animals, as shown by the parallel slopes of the dotted lines in fig. 1. ACTH-(4-10) only improved the retention of the acquired immobility caused by dexamethasone if it was administered chronically. Single injections of ACTH-(4-10) at 24 or 1 h before the initial test did not restore the corticosteroid effect (fig. 3).

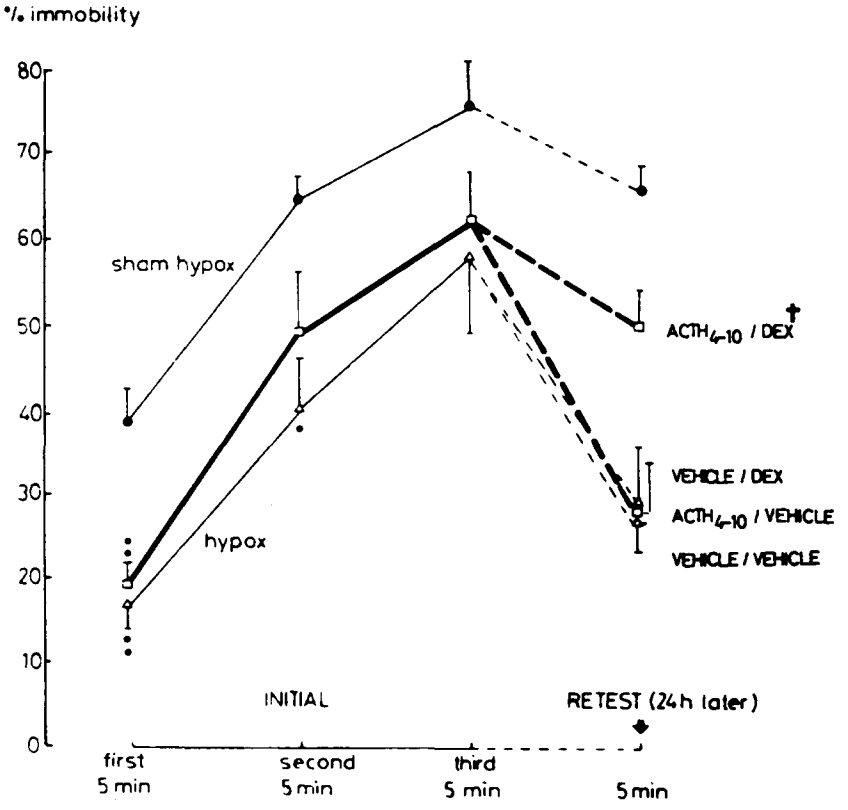

Fig. 1. Immobility of rats treated with ACTH-(4-10) (bold line □) or vehicle (normal line △) in the 15 min initial test (solid lines) and 5 min retest (24 h later, dotted lines) 2 weeks after hypophysectomy or sham operation-hypophysectomy (●). The chronic pretreatment as well as the dexamethasone or vehicle treatment after the initial test are indicated for each group. Acquisition of the immobility response in the initial test was determined in three intervals of five min each. Means ± S.E.M. are shown as percentages of the testing time. Initial test: $n = 6$ animals, retest: $n = 6$ animals per group. * $P < 0.05$, ** $P < 0.01$ vs. sham-operated, † $P < 0.05$ vs. sham-operated and $P < 0.01$ vs. vehicle/vehicle, vehicle/10 μg dexamethasone and ACTH-(4-10)/vehicle (Duncan's test).

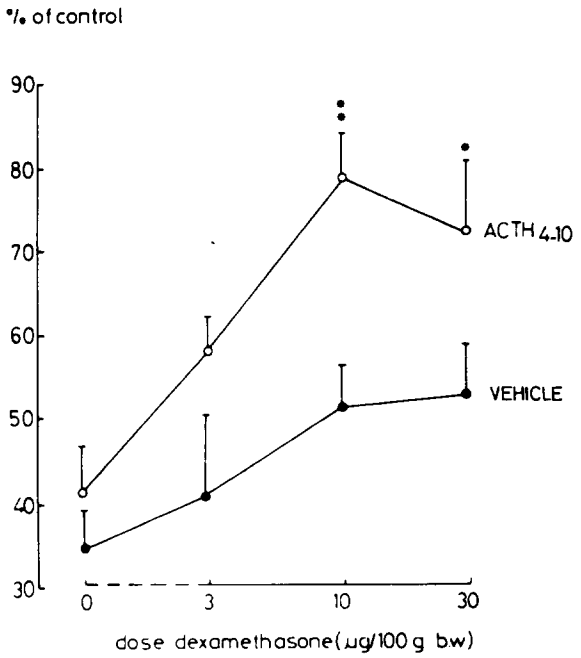

Fig. 2. Dose-dependent effect of s.c. administered dexamethasone on acquired immobility of ACTH-(4-10)- (○) or vehicle-(●) treated hypophysectomized animals (=100%). Data are expressed (means ± S.E.M.) as percentage of the immobility of sham-operated animals. n = 6 animals. * $P < 0.05$ vs. vehicle/30 µg dexamethasone, ** $P < 0.01$ vs. vehicle/10 µg dexamethasone (Duncan's test).

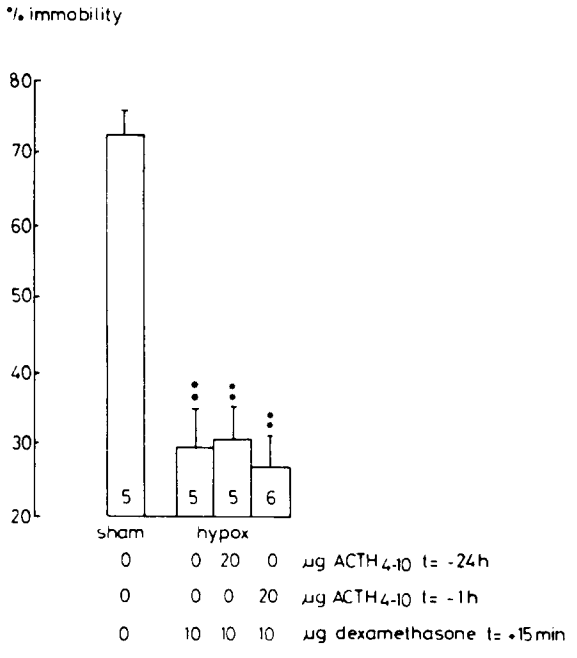

Fig. 3. Effect of 10 µg dexamethasone administered 15 min after the initial test to hypophysectomized (HYPOX) animals pretreated with a single ACTH-(4-10) injection at 24 h or 1 h before the initial test. Data are expressed as percentage of the testing time (means ± S.E.M.); number of rats is indicated in the column. ** $P < 0.01$ vs. sham-operated (Duncan's test).

4. Discussion

The study showed that hypophysectomized animals were deficient to retain the immobility they had acquired during the initial period of forced swimming if tested one or two weeks after surgery. This observation does not agree with the finding of Jefferys et al. (1983), who showed that retention by hypophysectomized animals was indistinguishable from that of sham-operated controls. We can offer no explanation for this discrepancy. The difference may be due to the shorter post-surgery interval in the Australian study (4-6 days) or could be related to the general physical and metabolic state of the hypophysectomized animals. In fact the poor performance of the hypophysectomized animals was used in our study to evidence the importance of the neurotrophic peptide in the expression of glucocorticoid effects on the brain. The deficiency in retention of the immobility response of the hypophysectomized animals could not be restored by administration of dexamethasone. The animals did however respond to dexamethasone after chronic pretreatment with the neurotrophic peptide ACTH-(4-10). A single injection of ACTH-(4-10) 24 h or 1 h before the initial test did not restore the steroid effect.

Jefferys et al. (1983; 1984; 1985) showed in several elegantly designed studies that adrenalectomized animals were deficient to retain the acquired immobility. This effect of adrenalectomy could be reversed by glucocorticoids as well as by opioid peptides. The authors postulated two alternate sensory pathways involving glucocorticoid and κ-opioid sensitive cells. It was also suggested

that activation of either pathway was sufficient to incorporate the immobility response via a common effector which is itself opioidergic, since naloxone antagonized both the glucocorticoid and the opioid reversal of the adrenalectomy-induced deficit. The κ-opioidergic mechanism was assumed to be situated peripherally. Our recent studies with intracerebral infusion of the antiglucocorticoid RU 38486 showed that glucocorticoids have a central site of action. The behavioural performance of intact animals was disturbed by the administration of 1 ng of the antiglucocorticoid RU 38486 in the dentate gyrus of the hippocampus (De Kloet et al., in press). The glucocorticoid effect is mediated by the Type 2 glucocorticoid receptor, since RU 38486 is an antagonist of this receptor (Moguilewsky and Philibert, 1983; Philibert, 1984). Moreover, the synthetic glucocorticoids dexamethasone and RU 28362 are much more potent than corticosterone to restore immobility (Veldhuis et al., 1985), which also indicates the involvement of the Type 2 receptor (Reul and De Kloet, 1985). Accordingly, the post-swimming rise of glucocorticoids and consequent glucocorticoid feedback via Type 2 terminates the stress response (Munck et al., 1984; Tausk, 1951; Tausk et al., 1986) and exerts a long-term effect on behavioural adaptation (Bohus et al., 1982, De Kloet and Reul, 1987).

The Type 2 receptors are indeed localized in abundance in hippocampal neurons but are also found in the paraventricular nucleus (where they regulate pituitary-adrenal activity) and in the ascending aminergic neurons (Fuxe et al., 1985; Van Eekelen et al., in press). In contrast the Type 1 (mineralocorticoid-like) receptor is almost exclusively localized in the hippocampus (McEwen et al., 1969; Reul and De Kloet, 1985) where it binds naturally occurring glucocorticoids with stringent specificity and thus acts as agonist in modulation of ongoing adaptive behaviour (De Kloet and Reul, 1987; De Kloet et al., 1986; McEwen et al., 1986).

ACTH-(4-10) is nearly devoid of the corticotrophic activity of the parent hormone ACTH but has maintained its behavioural activity. This neuroactive property of ACTH-(4-10) was discovered twenty years ago following administration of the peptide to hypophysectomized animals with the same dosage and timing scheme as used in the present study (De Wied, 1969). The peptide improved the shuttle box avoidance response, which was impaired in the hypophysectomized animals. Interestingly, thyroxine and testosterone exerted similar effects on the conditioned behaviour of hypophysectomized rats (De Wied, 1971) raising the possibility that the effects of the peptide were due to stimulation of nerve cell metabolism. ACTH-(4-10) was shown to delay the extinction of a conditioned avoidance response in intact animals while glucocorticoids facilitated extinction. The present study revealed another aspect of the interaction of hormones of the pituitary-adrenal system in the regulation of an adaptive response. ACTH-(4-10) appears to be a necessary requirement for the expression of glucocorticoid action on the brain mechanism that underlies the retention of acquired immobility. It is not yet known how such an ACTH-(4-10) action is achieved but there is evidence that this peptide-steroid interaction takes place at the steroid receptor level (Veldhuis and De Kloet, 1982). These studies and recent observations from this laboratory (Van Eekelen et al., unpublished observations) suggest that hypophysectomy converts the Type 2 receptor to an altered DNA binding state which can be reversed by ACTH-(4-10) treatment.

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