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INTRACEREBROVENTRICULAR ACTH ACTIVATES THE PITUITARY-ADRENAL SYSTEM:DISSOCIATION FROM A BEHAVIORAL RESPONSE

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Summary

In the rat, intracerebroventricular injection of synthetic ACTH ($ACTH_{1-24}$, $ACTH_{1-16}$) elevated plasma corticosterone levels and induced the display of excessive grooming behavior. The grooming response could be elicited in hypophysectomized rats without concommittant elevation of plasma corticosterone. In intact rats subcutaneous injection of $ACTH_{1-24}$ and not of $ACTH_{1-16}$-NH_2 stimulated the release of adrenal corticosteroids, whereas no excessive grooming was observed. In contrast to the reduced effectiveness of a second icv injection of ACTH in inducing the behavioral response, no single-dose tolerance was observed for the effect of icv ACTH on the pituitary-adrenal system. Therefore it was concluded that two different central mechanisms underly the observed responses to the icv applied ACTH.

Apart from its classical endocrine effects, the pituitary hormone ACTH exerts direct actions on the central nervous system, as was inferred from behavioral neurophysiological and neurochemical studies (1,2). Indeed there is accumulating evidence to suggest the presence of ACTH in the central nervous system. The peptide was demonstrated in cerebrospinal fluid (3) and in brain cells and structures (4,5). In addition to the existence of the peptidergic neurons containing ACTH, the presence of central ACTH may be accounted for by a possible ACTH transport mechanism from pituitary to septum (6). In the present communication we discuss evidence that intracerebroventricularly administered ACTH may modulate the hypothalamo-pituitary-adrenal axis independently from its induction of excessive grooming behavior in the rat.

Materials and Methods

Male rats of an inbred Wistar strain (TNO, Zeist, The Netherlands) were used, weighing approximately 150 g. Cannulation of the brain ventricular system at the level of the foramen interventriculare was performed at least five days prior to the experiment, essentially as described before (7). The animals were kept single-caged and were transported to a low noise room 24 h before the experiments. All peptides were dissolved in saline and injected into the ventricles in a final volume of 3 µl according to Brakkee *et al.* (7), or subcutaneously in a volume of 0.5 ml/100 g body weight. In one experiment the rats were hypophysectomized, using the transauricular route and completeness of the extirpation was verified by inspection of the sella turcica and was further evidenced by the

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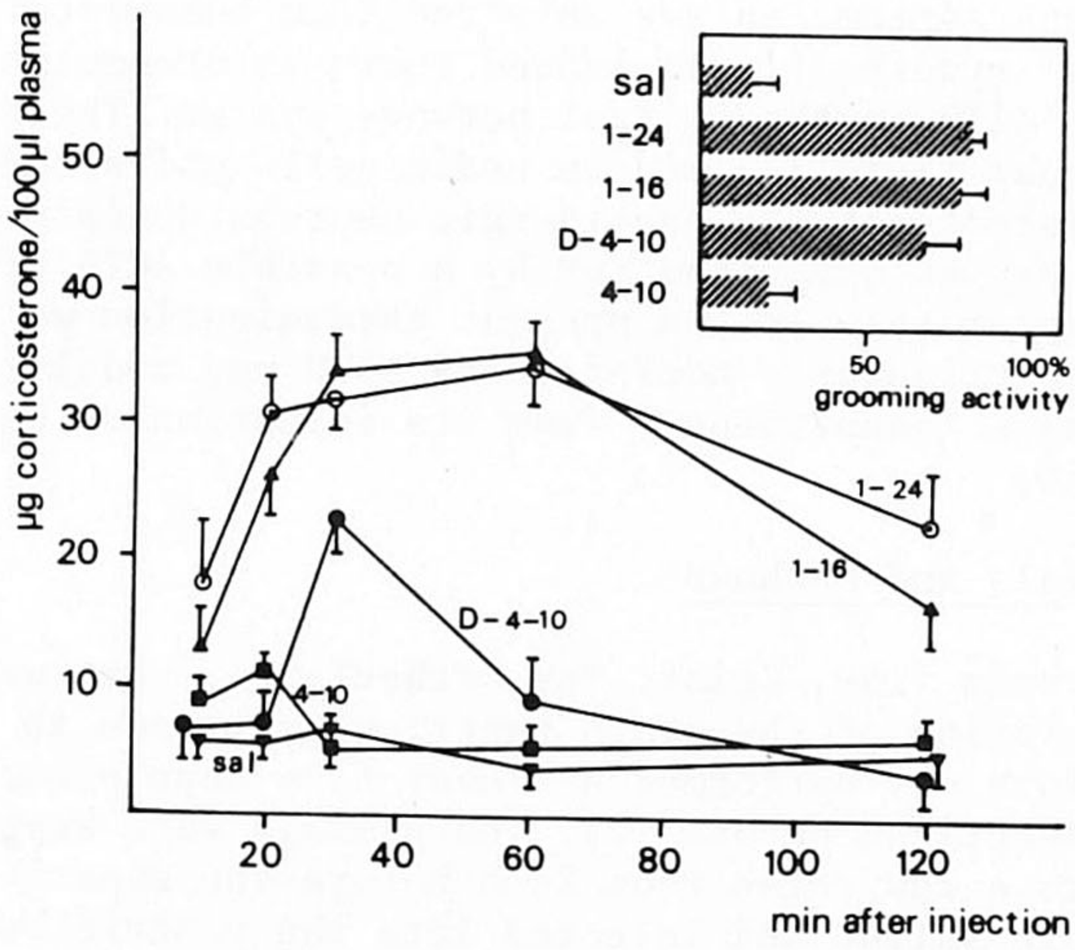
loss in body, adrenal and testicular weight. The occurrence of excessive grooming was recorded using a 15th second time sampling procedure essentially as described previously (8). At various times after the (icv or sc) injection(s) the animals were sacrificed by decapitation within 5 sec after removal from their cages. Blood from neck wounds was collected and plasma samples were assayed for corticosterone using the method of Murphy (9). All peptides used in this study were synthesized and kindly donated by Organon Int. B.V. (Oss, The Netherlands) and contained over 85% pure peptide.

Results

As shown in figure 1, intraventricular injection of 3 µl saline did not significantly influence the plasma concentration of corticosterone as measured 10, 20, 30, 60 and 120 min after the injection. If, however, $ACTH_{1-24}$ (1 µg/3 µl) or $ACTH_{1-16}$-NH_2 (1 µg/3 µl) was applied into the cerebral ventricles, a dramatic increase in plasma corticosterone levels was observed with a maximum around 30 to 60 min after the injection. Two hours after the injection still a significant elevation in plasma corticosterone was noted (Fig. 1). Icv injection of $ACTH_{4-10}$ (5 µg/3 µl) was ineffective, whereas |D-Phe^7|$ACTH_{4-10}$ (5 µg/3 µl) at 30 min after the injection initiated a rise in plasma corticosterone (Fig. 1). As was also described before (8), rats treated with icv injections of $ACTH_{1-24}$, $ACTH_{1-16}$-NH_2 or |D-Phe^7|$ACTH_{4-10}$ all displayed excessive grooming behavior which was absent in rats treated with saline or $ACTH_{4-10}$ (Fig. 1).

In order to test whether or not endogenous ACTH released from the pituitary was responsible for the observed rise in plasmacorticosteroids, in a next experiment hypophysectomized, cannulated rats, 24 h after removal of their pituitary, were used. Although an icv injection of $ACTH_{1-16}$-NH_2 (1 µg/3 µl)in these rats did induce the excessive grooming response (see also 8) a concommittant rise of plasma corticosterone could not be detected (Fig. 2), suggesting a mediating role of the pituitary in the activation of the adrenals. This suggestion was supported by findings on the effect of subcutaneous administration to intact rats of massive doses of $ACTH_{1-24}$ or $ACTH_{1-16}$-NH_2 on the adrenal steroid release. Only rats treated with $ACTH_{1-24}$ (50 µg/0.5 ml/rat) showed elevated plasma-corticosterone levels (Fig. 3). Rats treated with $ACTH_{1-16}$-NH_2 (65 µg/0.5 ml sc/rat)



▼—▼ 3 µl saline;
o—o $ACTH_{1-24}$ (1 µg);
▲—▲ $ACTH_{1-16}$-NH_2 (1 µg);
●—● |D-Phe^7|$ACTH_{4-10}$ (5 µg);
■—■ $ACTH_{4-10}$ (5 µg);

Excessive grooming was recorded for 1 h after the icv injection and expressed as percentage of maximal possible grooming score 240; see ref. 8). The elevation of plasma corticosterone in time for rats treated with $ACTH_{1-24}$, $ACTH_{1-16}$ or |D-Phe^7|$ACTH_{4-10}$ differed significantly from saline-treated rats ($p < 0.01$, repeated measures analysis of variance), n=5, mean values ± SEM.

Fig. 1. Effect of icv injected fragments of ACTH on plasma corticosterone and excessive grooming.

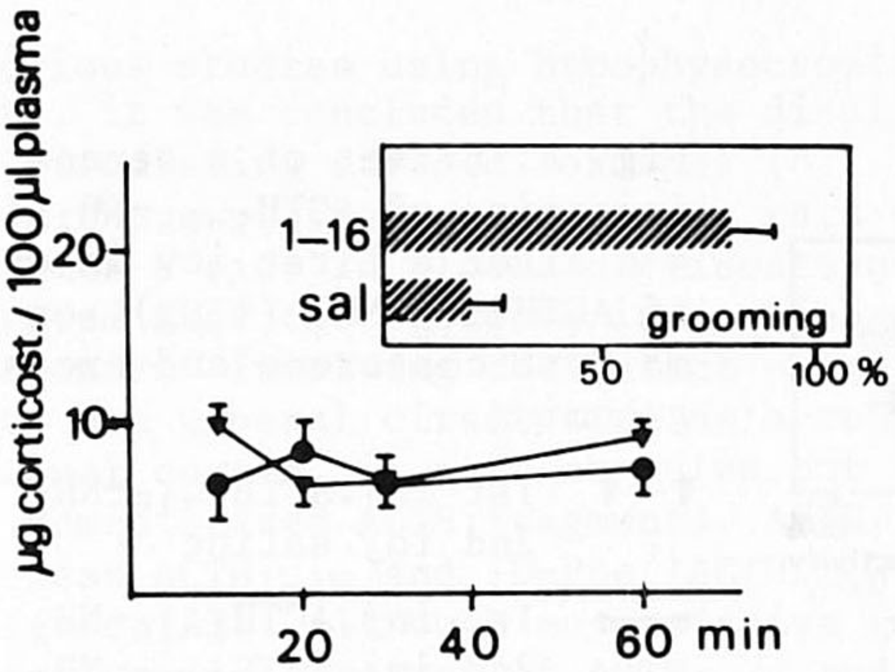


Fig. 2. Effect of icv injected $ACTH_{1-16}$-NH_2 (1 µg) on plasma corticosterone and excessive grooming in hypophysectomized rats.

●—● saline, n=5, mean values ± SEM;
▼—▼ $ACTH_{1-16}$, n=5;

did not differ from the saline-treated rats in this respect. Thus it would appear that a central mechanism underlies the peptide-initiated activation of the pituitary-adrenal system.Peripheral adminiministration of neither $ACTH_{1-24}$ nor $ACTH_{1-16}$-NH_2 induced excessive grooming (data not shown; see also 8).

An attempt was made to differentiate between the induction of excessive grooming and the stimulation of the steroid release. Therefore rats were injected icv at 10 a.m. with saline or $ACTH_{1-16}$-NH_2 and subsequently their grooming behavior was recorded in their home cages. At 2 p.m. all rats received a second icv injection with either saline or $ACTH_{1-16}$-NH_2 resulting in the following treatment groups: $ACTH_{1-16}$-NH_2/saline, $ACTH_{1-16}$-NH_2/$ACTH_{1-16}$-NH_2 and saline/$ACTH_{1-16}$-NH_2. Again their grooming behavior was recorded. Groups of rats were sacrificed 10, 30 or 60 min after this second icv injection and the levels of circulating plasma corticosterone were analyzed. As expected all rats with $ACTH_{1-16}$-NH_2 displayed excessive grooming behavior after the first administration of the peptide. However, as was reported before (10) 4 h later a second injection of ACTH was ineffective in producing this behavioral response (Fig.4). Yet, this second icv injection with $ACTH_{1-16}$-NH_2 irrespective of the prior treatments, significantly elevated plasma corticosterone levels, suggesting differences in neural substrates underlying peptide-induced excessive grooming and activation of the hypothalamo-pituitary-adrenal axis.

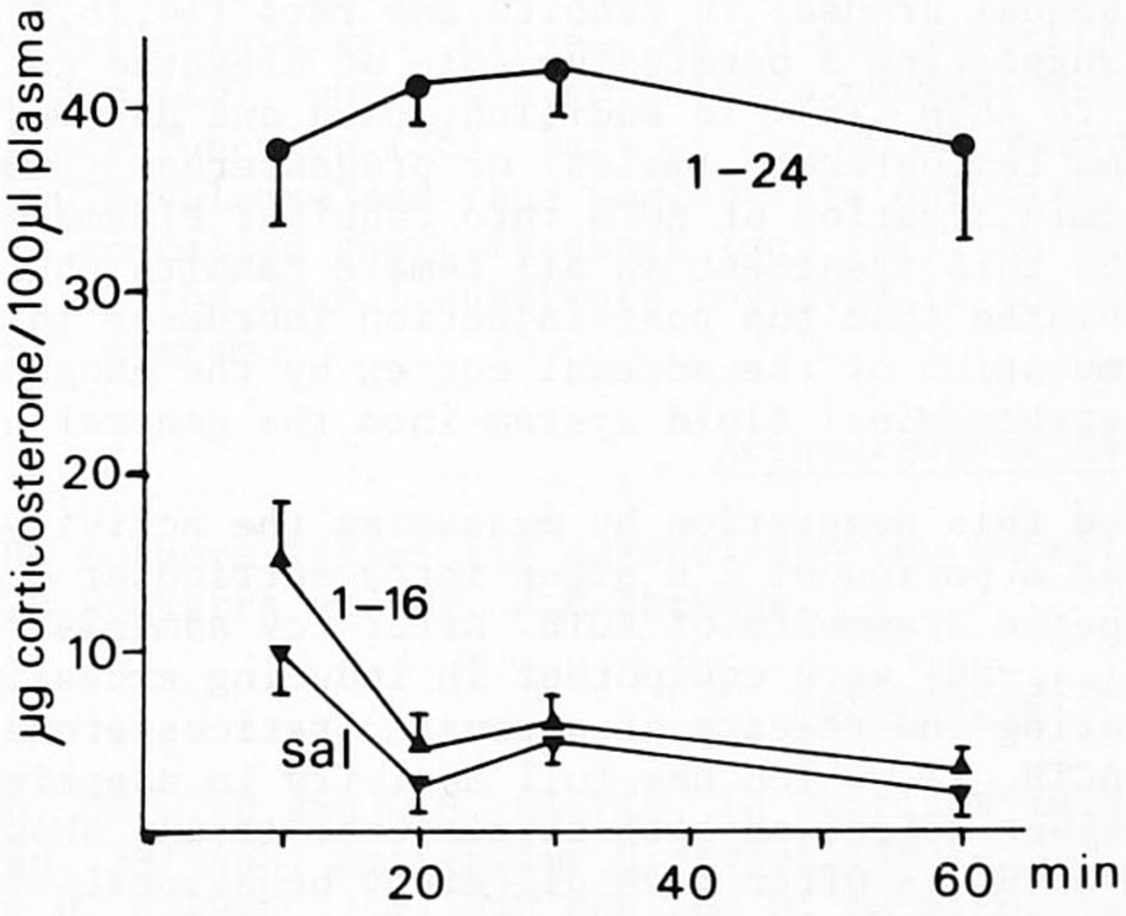


Fig. 3. Effect of subcutaneous injection of fragments of ACTH on plasma corticosterone.

▼—▼ saline(0.5 ml);
●—● $ACTH_{1-24}$(50 µg);
▲—▲ $ACTH_{1-16}$-NH_2(65 µg);
n=5,
mean values ± SEM.

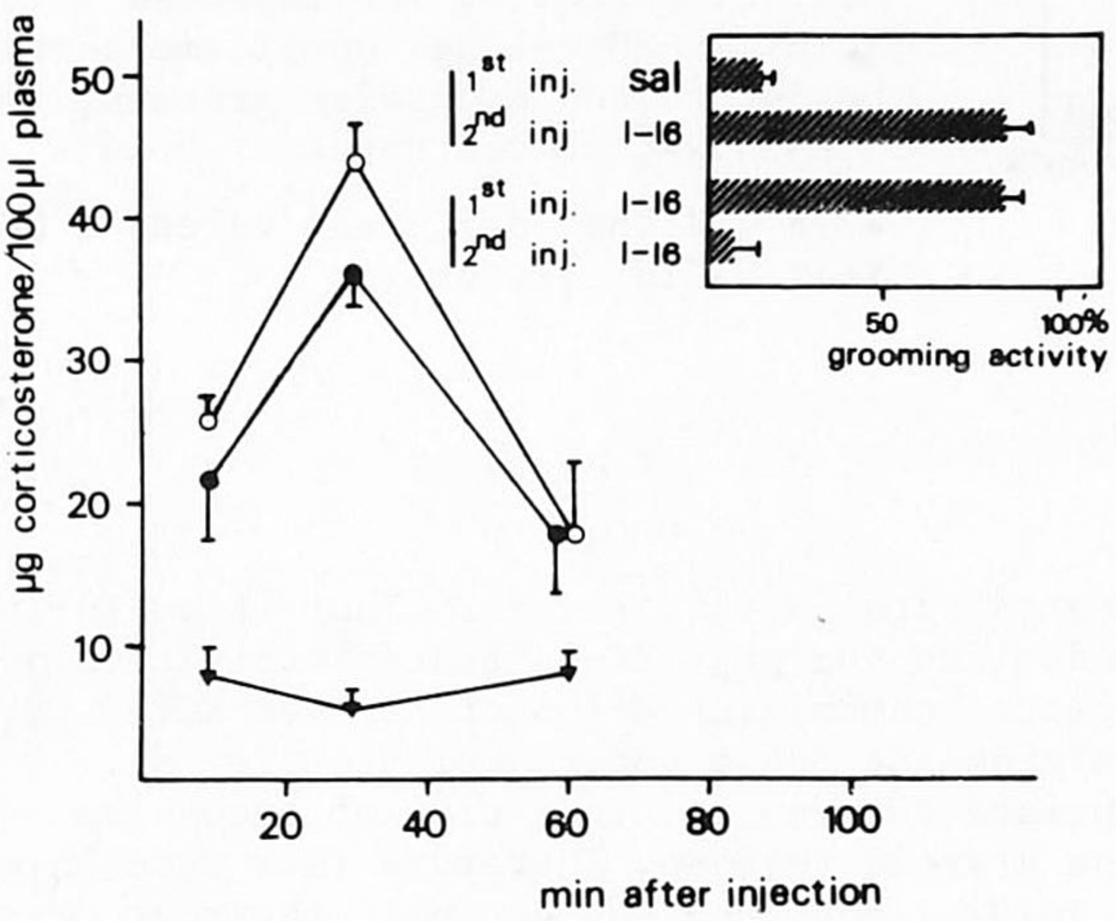


Fig. 4. Effect of a second icv injection of $ACTH_{1-16}$-NH_2(1 µg), 4 h after a first icv injection of $ACTH_{1-16}$-NH_2 (1 µg), on plasma corticosterone and excessive grooming.

▼—▼ 1st inj.$ACTH_{1-16}$-NH_2
2nd inj.saline

●—● 1st inj.$ACTH_{1-16}$-NH_2
2nd inj.$ACTH_{1-16}$-NH_2

○—○ 1st inj.saline
2nd inj.$ACTH_{1-16}$-NH_2

The elevation of plasma corticosterone in time for $ACTH_{1-16}$/$ACTH_{1-16}$ and saline/$ACTH_{1-16}$ differed from $ACTH_{1-16}$/saline ($p < 0.01$ repeated measures analysis of variance),n=5, mean values ± SEM.

Discussion

Intraventricular administration of peptides related to LPH, ACTH and MSH in rodents are known to induce a stretching and yawning syndrome (SYS) preceded by the display of excessive grooming (8,11,12,13). A variety of environmental variables is not able to influence the peptide-induced behavior. Only very strong motivational variables as severe hunger/thirst and anxiety are able to modulate the ACTH-initiated excessive grooming (14). Similarly Isaacson and Green (15) conclude that the effect of ACTH is not the enhancement of the most prominent response being made in a particular situation but that it is specific to grooming. Sofar the data on the relationship between ACTH and grooming are in line with the view that excessive grooming is a secondary response serving to dearouse the organism after activation by ACTH (14).

Some authors suggest that in addition to SYS intraventricular administration of these peptides also induced sexual arousal in rabbits and rats (14,16,17,18, 19,20). Evidence was obtained, suggesting a permissive role of elevated gonadal steroids in the sexual response to ACTH (18). In addition, Haun and Haltmeyer (19) showed an increase in plasma testosterone (males) or progesterone (females) after intercerebroventricular administration of ACTH into rabbits. Plasma corticosterone was also increased by this treatment in all female rabbits, but only in half of the males. They postulated that the post-injection increases in corticosterone seem to reflect stimulation of the adrenal cortex by the exogenous ACTH which "escaped" from the cerebrospinal fluid system into the general circulation (20).

In the present paper we tested this suggestion by measuring the activity of the pituitary adrenal system over a period of 2 h after intraventricular or parenteral administration of synthetic fragments of ACTH. After icv administration, the fragments $ACTH_{1-24}$ and $ACTH_{1-16}$-NH_2 were equipotent in inducing excessive grooming behavior and in stimulating the release of adrenal corsticosterone (Fig. 1). The smaller fragment $ACTH_{4-10}$, which has full activity in adaptive behavioral processes (1) was without effect on both parameters. As was shown previously $|D\text{-}Phe^7|ACTH_{4-10}$ and $ACTH_{4-10}$ often have different behavioral effects (21,22), and indeed the D-enantiomer induced considerable display of excessive grooming and a small but significant rise in plasma corticosterone levels. Thus, it seems that if a peptide has grooming-inducing potency, intraventricular administration leads to an enhanced release of adrenal corticosterone. From

previous studies using hypophysectomized, adrenalectomized or gonadectomized rats, it was concluded that the display of excessive grooming was not dependent on circulating steroid hormones (8). This conclusion is supported by the data from the experiment using hypox rats which did show excessive grooming behavior without a rise in plasma corticosterone (Fig. 2). This experiment further demonstrated that the increase in corticosterone after icv ACTH is brought about by a mechanism, involving the intact pituitary. Leakage from the cerebrospinal fluid into the general circulation as a route by which icv ACTH could activate the adrenal cortex, is further ruled out by the differential effect of subcutaneously administered ACTH fragments. $ACTH_{1-24}$ has full corticotrophic activity, whereas $ACTH_{1-16}$ and $|D\text{-}Phe^{7}|ACTH_{4-10}$ do not stimulate adrenal cortical steroid release (23). Although no excessive grooming behavior was observed after peripheral administration of $ACTH_{1-24}$ and $ACTH_{1-16}$, in the case of $ACTH_{1-24}$ treatment a dramatic rise in plasma corticosterone level was observed (Fig. 3).

The last experiment pertained to the question what if any relation exists between the effect of ACTH on grooming and on the pituitary-adrenal axis. It could be envisaged that the behavioral response induced by the peptide, would trigger the activation of the endocrine axis. ACTH was equally active in adrenalectomized and intact rats (8), so it is unlikely that the rise in plasma steroid levels is responsible for the induction of excessive grooming. Another explanation of the data sofar, could be that ACTH independently activates two neural substrates, one responsible for the effects on grooming behavior, and the other involved in the regulation of the pituitary function. As was shown before there is good evidence that there is a refractory period in which a second injection of peptide is remarkably ineffective in producing the behavioral response. Detailed analysis of this phenomenon led us to presume that most likely the neural substrate temporarily had become tolerant to the peptide (10,24). This property of the grooming response was used to study the relation of the two effects brought about by central application of ACTH. It was found that a second injection of ACTH 4 h after a first administration, is still a strong stimulus for the activation of the pituitary-adrenal system despite its ineffectivity to produce the behavioral response. In view of this differential effect on hormone release and grooming behavior, it is suggested that the two responses are brought about by two different interactions of ACTH and the central nervous system. Such a conclusion is in accordance with the working hypothesis previously formulated, describing the central effects of ACTH in terms of multiple neural substrates with possibly multiple receptors (2).

With respect to the onset of excessive grooming the dopaminergic neurons in the substantia nigra projecting in the neostriatum and nucleus accumbens, are important (25), whereas hypothalamic neurons are involved in the control of the pituitary function (26). Part of the latter neurons may be sensitive to ACTH,enhancing ACTH release and thus may operate opposite to those inhibiting ACTH-release by the so-called short feedback loop (27). Further work is in progress to characterize the neural substrate involved in the ACTH stimulation of the pituitary-adrenal system.

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