

THE HIPPOCAMPAL CORTICOSTERONE RECEPTOR SYSTEM OF THE HOMOZYGOUS DIABETES INSIPIDUS (BRATTLEBORO) RAT

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SUMMARY

The binding of [^3H] corticosterone to hippocampal cytosol receptors of Brattleboro rats homozygous for diabetes insipidus (Ho-Di) and of normal Brattleboro rats (Ho-No) was investigated at 24 h after removal of the adrenals. The apparent maximal binding capacity of the Ho-Di hippocampal corticosterone receptor system was about 30% less than that of the Ho-No rats. Substitution of the vasopressin deficient rats with 1E pitressin tannate in oil partially restores the hippocampal corticosterone receptor level towards that of the control animals.

The rat brain and in particular the hippocampus contains receptor sites for corticosterone, which is the principal adrenal glucocorticoid secreted by the adrenal cortex [9,13,15]. The hippocampal corticosterone receptor system is implicated in the physiological control of adaptive behavior [1,3,4]. Peptides related to ACTH, the endorphins, and the neurohypophyseal hormones have profound effects on learning and memory processes [8,23]. Such neuro-peptides and corticosterone represent different but interacting hormonal systems involved in the control of these particular brain functions [4,22]. The concept of hormonal control of adaptive behavior has emerged from a classical endocrine approach: removal of the endocrine gland (pituitary or adrenal) or a genetically determined lack of a particular hormone produced a behavioral disturbance, which could be restored by appropriate hormone replacement [21]. Essentially this approach was used in the present study to analyze the action of exogenous vasopressin on the hippocampal corticosterone receptor system. The experiments were performed with homozygous diabetes insipidus rats (Ho-Di) of the Brattleboro strain, which have a hereditary lack in the synthesis of vasopressin [20].

Adult male Ho-Di rats (200 g) were substituted for one week daily with

1E pitressin tannate in oil (a lysine vasopressin enriched porcine pituitary extract, Parke Davis Co.) subcutaneously; the last administration 24 h prior to sacrifice. Ho-Di, pitressin tannate substituted Ho-Di and normal homozygous Brattleboro rats (Ho-No) were adrenalectomized bilaterally. Twenty-four hours later the rats were perfused under Nembutal anesthesia via the heart with saline. Hippocampal tissue was removed from the brain on ice [6], homogenized, centrifuged and assayed for [3 H] corticosterone binding in cytosol as previously described [9]. The medium was 5 mM Tris buffer (Tris(hydroxymethyl)aminomethane) containing 1 mM EDTA (ethylene diamine tetra acetate, disodium salt), 1 mM 2 mercaptoethanol and 5% glycerol adjusted to pH = 7.4 with hydrochloric acid. [3 H] Corticosterone (spec. act., 50 Ci/mM, New England Nuclear) was added to the cytosol giving concentrations ranging from $1 \cdot 10^{-10}$ M up to $2 \cdot 10^{-8}$ M. Aspecific binding was determined in parallel incubations including a five hundred fold excess of unlabelled steroid. In another series of experiments using male Wistar rats (± 200 g) the binding of [3 H] corticosterone ($2 \cdot 10^{-8}$ M) was measured at various times after adrenalectomy. In these experiments the removal of the adrenals occurred either in the morning or in the evening.

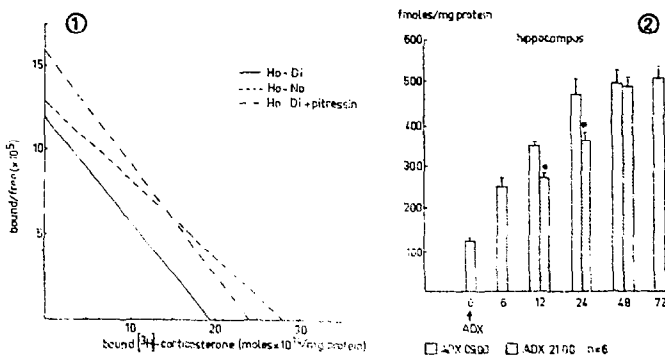


Fig. 1. Scatchard plot of binding of [3 H] corticosterone to hippocampal cytosol receptors. Scatchard analysis was based on data of 10 separate binding experiments. Each binding assay was performed with [3 H] corticosterone concentrations ranging from $1 \cdot 10^{-10}$ M up to $2 \cdot 10^{-8}$ M. Aspecific binding was determined in parallel series of incubations including a five hundred fold excess of unlabelled corticosterone. Apparent binding constants: Ho-Di: $B_{\max} = 194 \pm 17$ fmol/mg protein, $K_d = 1.61 \cdot 10^{-9}$ M ($r_{\text{corr}} = 0.99$); Ho-No: $B_{\max} = 279 \pm 16$ fmol/mg protein, $K_d = 2.16 \cdot 10^{-9}$ M ($r_{\text{corr}} = 0.98$); Ho-Di + pitressin: $B_{\max} = 240 \pm 18$ fmol/mg protein, $K_d = 1.51 \cdot 10^{-9}$ M ($r_{\text{corr}} = 0.99$).

Fig. 2. Binding of [3 H] corticosterone ($2 \cdot 10^{-8}$ M) to hippocampal cytosol receptors at various times after adrenalectomy. Adrenals were removed either in the morning (09:00) or evening (21:00). $t=0$ is the binding in intact animals maintained until sacrifice under non-stressful conditions. Data are average of $n=6$ animals \pm S.E.M.

Figure 1 shows that the apparent maximal binding capacity calculated after analyses of the binding data via a Scatchard plot is approx. 30% less for Ho-Di ($B_{\max} = 194$ fmol/mg protein) than for Ho-No rats ($B_{\max} = 279$ fmol/mg protein). Daily administration of 1E pitressin tannate in oil for one week to Ho-Di rats caused a significantly increased binding capacity to a level between that of Ho-Di and Ho-No rats ($B_{\max} = 240$ fmol/mg protein). The affinity constants for the corticosterone receptor binding did not change after pitressin administration ($K_d = 1.5-1.6 \cdot 10^{-9}$ M) but remained lower than that of Ho-No animals ($K_d = 2.2 \cdot 10^{-9}$ M). The tissues were removed from rats, which were adrenalectomized 24 h previously in order to deplete the hippocampal corticosterone receptor system of endogenous hormone. Figure 2 shows for Wistar rats that as a function of time there is a rapid increase in available binding sites reaching a plateau after 24 h. Between 24 and 72 h after adrenalectomy the binding increased only for about 10%. Removal of the adrenal in the evening (21:00), when plasma corticosterone levels are high, resulted after 24 h in a 25% lower [3 H]corticosterone binding than when adrenalectomy was performed in the morning (09:00). The circadian difference in amount of receptors gradually disappeared the next day.

The present study shows that the capacity of the hippocampal corticosterone receptor system is substantially less for Ho-Di than for Ho-No rats. This observation was made 24 h after adrenalectomy. Removal of the adrenals results in an increase in receptor sites available for binding of [3 H]-corticosterone and the binding reaches near maximal levels after 24 h. This increase in available binding sites is in the first place due to the disappearance of endogenous corticosterone during the first hours and possibly later on representative for newly synthesized receptor protein. In contrast to another report [14] the increased binding after adrenalectomy appeared not a biphasic, but a continuous process. The discrepancy may be related to pre-surgical plasma corticosterone levels; the circadian variation in the amount of receptor sites measured up to 24 h after adrenalectomy is consonant with this notion (Fig. 2 and Ref. 18). Chronic corticosterone administration via the drinking water or chronic stress cause decreased hippocampal corticosterone receptor levels up to 4 days after adrenalectomy [19]. Dominant mice have a less active pituitary-adrenal system [5] than subordinates and also have an enhanced tissue uptake of [3 H]corticosterone, which is indicative for a larger amount of corticosterone receptors [19]. These observations suggest, that the amount of corticosterone receptors, measured after adrenalectomy, primarily is under control of presurgical plasma concentrations of the proper hormone. The reduced post-adrenalectomy increase in [3 H]corticosterone binding observed in Ho-Di rats may be caused by a slower rate of disappearance of endogenous hormone. The metabolic half life of corticosterone in plasma of Ho-Di rats is, however, shorter than in that of Ho-No (12.7 min for Ho-Di and 19.5 min for Ho-No; de Kloet, unpublished observation). In addition, Ho-Di rats have in general a reduced adrenal cortico-

sterone secretion [17]. Therefore, the reduced post-adrenalectomy hippocampal binding capacity of Ho-Di rats rather seems related to a smaller pre-adrenalectomy binding capacity of the receptor system. Present methodology does not allow, however, to estimate the binding capacity in intact rats due to variability of receptor occupation with endogenous hormone.

This study shows that pitressin augments the reduced amount of corticosterone receptors in Ho-Di rats. Such an action of the vasopressin enriched porcine pituitary extract may be secondarily to peripheral changes effectuated by the hormone treatment. The dose is sufficient to restore the abnormal water balance of these rats. Chronic pitressin treatment has been reported to increase partially the hypothalamic CRH content and pituitary ACTH responsiveness of Ho-Di rats as well as adrenal corticosterone secretion towards the level of normal animals [10,11,17]. It could be argued that the enhanced adrenal corticosterone concentration influences the post-adrenalectomy increase in amount of hippocampal corticosterone receptors. However, elevated plasma corticosterone levels would diminish the capacity of the binding system, while pitressin treatment results in the opposite effect (Fig. 2 and Ref. 19).

Ho-Di rats have in general a hypo-active endocrine system [2,12,16,17] and the reduced amount of receptors may be a reflection of the genetic difference between the two strains. There are, however, arguments to assign a specific role to vasopressin in the effect on neuronal processes involved in corticosterone action. A recent study from this laboratory showed that a single vasopressin injection restores a severe memory deficit of diabetes insipidus rats [25], but the pituitary-adrenal response accompanying the behavioral response in normal rats cannot be altered by this treatment [24]. The same observation was made with des¹-glycinamide³-arginine vasopressin (DG-AVP) a peptide lacking the classical endocrine effects of vasopressin, but maintaining the behavioral activity [26]. Preliminary observations have shown that chronic administration of DG-AVP also restores the diminished receptor capacity for corticosterone in the hippocampus of Ho-Di rats (Veldhuis and de Kloet, in preprint). It is, therefore, conceivable that the vasopressin action is related to the neuropeptide rather than their endocrine properties [7]. Accordingly, hippocampal receptor activity for corticosterone is not only under control of the proper hormone, but also seems affected by peptides such as vasopressin.

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