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Binding of corticosteroid receptors to rat hippocampus nuclear matrix

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In rat hippocampus, the mineralocorticoid receptor and the glucocorticoid receptor bind corticosterone with high affinity. We have studied the association of these receptors with the nuclear matrix both after in vivo and in vitro administration of radiolabelled corticosterone to hippocampus cells. It was found that in vivo 100% and in vitro 60% of the corticosterone that specifically bound to rat hippocampus nuclei was attached to the nuclear matrix. A selective glucocorticoid receptor agonist did not compete for corticosterone binding. This indicates that this binding was mediated by the mineralocorticoid receptor rather than the glucocorticoid receptor.

Glucocorticoid receptor; Mineralocorticoid receptor; Corticosterone; Nuclear matrix; Association; Rat hippocampus

1. INTRODUCTION

In rat hippocampus, two types of steroid receptors have been identified which show high affinity for corticosterone (CORT), the major glucocorticoid in the rat. These are the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [1]. The two types of receptors are assumed to play a key role in controlling the neuroendocrine responses to stress [2–4].

Several members of the steroid receptor superfamily [5] have been shown to interact with the nuclear matrix, a putative skeleton-like structure in the cell nucleus [6]. The nuclear matrix is operationally defined as the nuclear fraction that remains after extraction of the chromatin and all soluble and loosely-bound components [7]. It is the site of action of many important nuclear processes, such as transcription [8], replication [9], RNA processing [10] and RNA transport [11].

We report here the binding of radiolabelled CORT to the nuclear matrix of rat hippocampus cells under in vivo and in vitro conditions. The contribution of the MR and the GR to the binding of labelled CORT to the nuclear matrix was investigated in vitro by competition studies with RU28362, a glucocorticoid receptor-selective steroid. The results indicate that the MR is associated with the nuclear matrix, whereas no binding of the GR could be detected.

Abbreviations: GR, glucocorticoid receptor; MR, mineralocorticoid receptor; CORT, corticosterone; ADX, adrenalectomized.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

DNaseI and RNase were obtained from Boehringer-Mannheim, Germany. Sodium tetrathionate (NaTT) and phenylmethylsulphonylfluoride (PMSF) were from Sigma. ^{3}H-Labelled corticosterone (80–100 Ci/mmol) was from New England Nuclear, Dreieich, Germany. Unlabelled corticosterone was a gift from Organon, Oss, The Netherlands. Unlabelled RU28362 was provided by Roussel-Uclaf, France.

2.2. *Animals and surgery*

Male Wistar rats were housed under standard conditions and given food (standard lab chow) and water ad libitum. Rats weighing 180–250 g were adrenalectomized (ADX) under ether anaesthesia via the dorsal approach, 3–5 days before the experiment. After adrenalectomy, 0.9% NaCl was added to the drinking water.

2.3. *In vivo steroid binding*

ADX rats were injected subcutaneously with 0.5 ml of 40% ethanol/saline, with or without 1 mg of unlabelled CORT, to determine non-specific binding. After 30 min 50 μCi [^{3}H]CORT in 0.2 ml 25% ethanol/saline was injected into the tail vein. Sixty minutes later the rats were killed by decapitation, the brainwas removed from the skull and placed on an ice-chilled plate. The hippocampi were dissected rapidly, immediately frozen on dry-ice and stored at −80°C for a maximum of one week. Blood was collected to check serum concentrations of radiolabelled hormone.

2.4. *In vitro steroid binding*

The experimental design for in vitro binding studies was as described previously [12]. ADX rats were sacrificed by decapitation and the brain was immediately removed from the skull and placed on an ice-chilled plate. The hippocampus was rapidly dissected and chopped to 300 μm slices, using a McIlwain tissue chopper. Slices from two or three hippocampi were pooled and incubated for 1 h at 25°C with 18–24 nM of [^{3}H]CORT, in 4 ml Krebs-Ringer buffer saturated with 95% O_2/5% CO_2. Non-specific steroid binding was determined by incubation in the presence of a 500-fold excess of unlabelled CORT. In some experiments, incubations included a 100-fold excess of unlabelled RU28362.

2.5. *Isolation of cell nuclei*

Nuclei were isolated essentially as described by Kaufmann et al.

[13]. To all buffers 0.2 mM PMSF was added from a 100 mM stock solution in 100% ethanol. The entire isolation was carried out on ice or at 4°C. Pooled hippocampi from 2 or 3 rats were homogenized in 9 ml STM buffer (250 mM sucrose, 50 mM Tris-HCl, 5 mM $MgSO_4$, pH 7.4) in a 10 ml Potter Elvejhem homogenisation tube with a teflon pestle (10 strokes at 500 rpm followed by 5 strokes at 1000 rpm). The homogenate was centrifuged 15 min at $800 \times g$. The pellet was carefully resuspended in 10 ml STM and centrifuged 15 min at $800 \times g$. The pellet was resuspended in 2.2 or 3.4 ml DS buffer (1.95 M sucrose, 50 mM Tris-HCl, 5 mM $MgSO_4$, pH 7.4) and centrifuged 60 min at $72\,000 \times g$ in a swing-out rotor. The final pellet consisted of approximately 10^7 purified nuclei.

2.6. *Isolation of nuclear matrices*

Nuclear matrices were isolated essentially as described by Kaufmann et al. [13]. To all buffers PMSF was added to a concentration of 1 mM prior to use. To avoid mechanical disruption of the nuclei or nuclear matrices by shearing forces, pellets were gently resuspended using a 200 μl pipette with disposable tips from which 3 mm was cut to enlarge the opening diameter. The entire isolation was carried out on ice.

The nuclear pellet was resuspended in 400 μl STM/0.5 mM NaTT. From this fraction a 70 μl sample was taken to determine the steroid binding and the number of nuclei. After 60 min incubation in STM/0.5 mM NaTT, the nuclei were washed three times by repeated resuspension and sedimentation (5 min at $4000 \times g$) in 400 μl STM. The pellet was then resuspended and incubated 60 min in 400 μl STM, containing 250 μg DNase I and 250 μg RNase. After spinning down, the pellet was resuspended in 75 μl LS buffer (10 mM Tris-HCl, 0.2 mM $MgSO_4$, pH 7.4). While stirring continuously on a Vortex mixer at low speed, 300 μl HS buffer (2 M NaCl in LS buffer) was added slowly, followed by 10 min incubation. This LS/HS extraction was repeated once. The resulting nuclear matrices were sedimented (10 min, $8000 \times g$) and resuspended in 200 μl STM. Steroid binding and the number of nuclear matrices in this final fraction were determined. About $2\text{–}4 \times 10^6$ nuclear matrices were thus obtained.

2.7. *Quantification of steroid binding*

Radioactivity of nuclear and nuclear matrix samples was measured in a Hewlet Packard TriCarb scintillation counter, using 4 ml Opti-Fluor scintillation fluid per sample in mini vials. The number of nuclei and nuclear matrices was determined with a Bürker counting chamber.

3. RESULTS

In vivo binding of CORT to hippocampus nuclear matrices was studied by injection of ADX rats with radiolabelled CORT, followed one hour later by dissection of the hippocampus, and subsequent isolation of nuclei and nuclear matrices. Injection of 50 μCi [^{3}H]CORT in ADX rats resulted in a total (free plus transcortin-bound [^{3}H]CORT) serum concentration of about 1.5 nM. Fig. 1 shows that the steroid was bound specifically to the nuclear and nuclear matrix fractions from hippocampus tissue. Approximately all of the [^{3}H]CORT bound to the nuclei after in vivo injection was recovered in the nuclear matrix fraction, indicating that most of the nuclear [^{3}H]CORT binding sites were nuclear matrix-associated. Binding of [^{3}H]CORT to the nuclear matrix was very stable, since no bound steroid was lost during the nuclear matrix isolation procedure, which took about 5 h. Furthermore, binding was resistant to treatment with low and high ionic strength buffers.

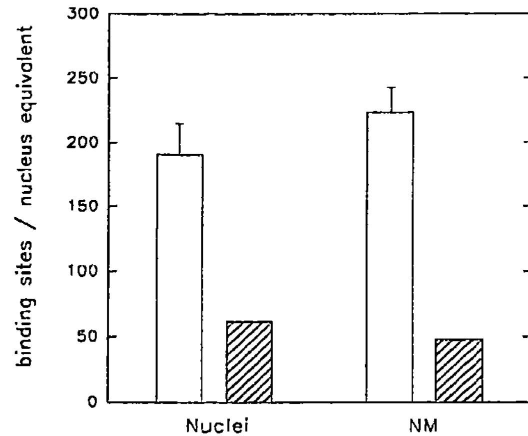

Fig. 1. In vivo binding (mean ± SE) of [^{3}H]CORT to hippocampus cell nuclei and nuclear matrices (NM). Open bars, total binding (n=3); hatched bars, non-specific binding (n=1).

To identify the CORT-binding sites in the nuclear matrix fraction, an in vitro method was used in which 300 μm hippocampal slices were incubated at 25°C with steroid in carbogenated Krebs-Ringer buffer. This method has previously been used for studying nuclear uptake of steroids in neural tissue [12]. As displayed in Fig. 2, in vitro incubation of hippocampus tissue slices with about 20 nM [^{3}H]CORT resulted in specific binding of the labelled steroid to the subsequently prepared nuclear and nuclear matrix fraction. The number of detected binding sites per nucleus or nuclear matrix was about 30 times higher than in the in vivo experiment, probably due to a much higher intracellular concentration of [^{3}H]CORT. Unlike in the in vivo experiment, only about 60% of the nuclear binding was recovered in the nuclear matrix fraction.

The in vitro method was used to study the contribution of the GR to the CORT binding to the nuclear matrices. Hippocampus slices were incubated with [^{3}H]CORT in the absence or presence of a 100-fold excess of unlabelled RU28362, a synthetic steroid which

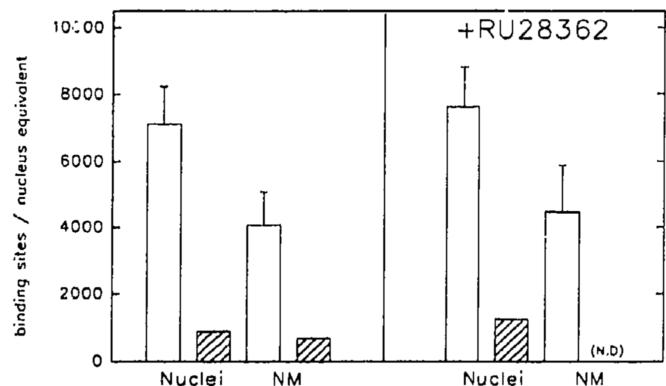

Fig. 2. In vitro binding (mean ± SE) of [^{3}H]CORT to cell nuclei and nuclear matrices (NM) in hippocampal tissue slices. Left panel, incubation of slices with [^{3}H]CORT. Right panel, incubation of slices with [^{3}H]CORT in the presence of a 100-fold excess RU28362. Open bars, total binding (n=4 or 5). Hatched bars, non-specific binding (n=1).

selectively occupies the GR (Fig. 2). No change in [^{3}H]CORT binding, either to nuclei or to nuclear matrices, could be observed when excess RU28362 was added, indicating that the GR is not involved in the observed association of [^{3}H]CORT to nuclei and nuclear matrices.

4. DISCUSSION

The data presented here show that CORT is associated with the nuclear matrix after in vivo or in vitro administration to hippocampus cells. Although it cannot be excluded that an unknown binding site mediates the binding of CORT to the nuclear matrix, the absence of competition by a 100-fold excess of RU28362 in vitro strongly suggests that the MR is bound to the nuclear matrix.

Previous reports have described the association of several members of the steroid receptor superfamily with the nuclear matrix, including the estrogen receptor in rat liver and uterus [14,15], the androgen receptor in rat prostate [16], the thyroid hormone receptor in a rat pituitary tumour cell line [17], and the GR in rat liver [13,18]. The data shown here provide evidence that this list can be extended with the MR in rat hippocampus. It is conceivable that interaction with the nuclear matrix is a common feature of all members of the steroid receptor superfamily.

Since not only the MR, but also the GR in the rat hippocampus shows a high affinity for CORT (K_d = 0.5 and 2–5 nM, respectively [1]), it can be assumed that at the applied in vitro concentration (approximately 20 nM), [^{3}H]CORT binds to both GR and MR. This raises the question of why association of CORT with the nuclei and nuclear matrices shows only MR specificity. One explanation is that [^{3}H]CORT has dissociated from the GR during the isolation procedure, but not from the MR. This is supported by the observation that in hippocampus cytosol preparations the dissociation rate of the GR–CORT complex is much higher than that of the MR–CORT complex ($t_{1/2}$) = 2.2 h and 34 h, respectively) [19]. Alternatively the GR–CORT complex may have no, or only a very weak, interaction with nuclear components in this in vitro system. Kaufmann et al. [13] have demonstrated the presence of the GR in rat liver nuclear matrices, using [^{3}H]triamcinolone acetonide. This suggests that the binding of the GR to the nuclear matrix is tissue-specific, or depends on the ligand that is used.

The physiological significance of the interaction of steroid receptors with the nuclear matrix is not known. The binding of steroid receptors to the nuclear matrix has been shown to occur only after hormone treatment [13,15], and to be correlated with the expression of specific steroid-responsive genes [14]. Other transcription factors [20], as well as transcriptionally active genes [21] and RNA polymerase II [8], are attached to the nuclear matrix. Steroid-responsive genes also become nuclear matrix-associated after stimulation with the appropriate hormone, and dissociate from the matrix upon hormone withdrawal [22]. Together, these data strongly suggest that transcription complexes are intimately associated with the nuclear matrix. The importance of the binding of steroid receptors to the nuclear matrix for the regulation of steroid hormone-responsive genes remains to be elucidated.

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