Journal of Steroid Biochemistry, Vol. 11, pp. 845 to 854
Pergamon Press Ltd 1979. Printed in Great Britain

NUCLEAR PROGESTERONE RECEPTORS AND CHARACTERIZATION OF CYTOSOL RECEPTORS IN THE RAT HYPOTHALAMUS AND ANTERIOR HYPOPHYSIS

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SUMMARY

Progesterone receptors in nuclei and cytosols from the hypothalamus and anterior hypophysis of oestrogen-primed immature and mature female rats were investigated. In the hypothalamic and hypophysial nuclei the binding and exchange of [^{3}H]-R5020 with progesterone or R5020 was achieved after 2 h at 0–10°C, but rapidly degraded at 30°C. In addition, when unlabelled R5020 was added to the incubation tubes previously incubated with [^{3}H]-R5020 at 0–10°C, unlabelled R5020 was found to exchange with [^{3}H]-R5020 bound to nuclei, confirming that [^{3}H]-R5020 binding is due to an exchange reaction. Scatchard analysis of the specific binding curves revealed high-affinity and low capacity binding. Progesterone receptor complexes extracted with 0.4 M KCl from purified and crude (800 *g* pellet) nuclei prepared from the hypothalamus and anterior hypophysis of the oestrogen-primed adult female rats incubated with [^{3}H]-R5020 were identified in the vicinity of 5S by gradient centrifugation. From these results it is concluded that nuclear progesterone receptors exist in the hypothalamus and anterior hypophysis. Moreover, it is interesting to note that progestin binding sites resistant to extraction with 0.4 M KCl exist even in the purified hypothalamic and hypophysial nuclei.

In the hypothalamic and anterior hypophysial cytosols an exchange reaction was observed at 0–10°C as in the nuclei. The 7S cytosol receptors at low ionic strength sedimented in the 4S region in a high salt medium (0.4 M KCl), both in the hypothalamus or hypophysis, suggesting a possible relationship between aggregate- and subunit receptors. Moreover, progesterone receptors in the hypothalamic and hypophysial cytosols were separated on polyacrylamide agarose gels electrophoretically from oestrogen- and androgen-receptors labelled with [^{3}H]-R2858 and [^{3}H]-R1881, respectively.

The existence of nuclear progesterone receptors in the hypothalamus and anterior hypophysis, together with the cytosol receptors, provide further evidence for a possible role of the steroid–receptor interaction in the mechanism of the central action of progesterone.

INTRODUCTION

The isolation of specific progestin binding receptor macromolecule proteins by gradient centrifugation from hypothalamic and hypophysial cytosols of oestrogen-primed immature and mature female rats [1–3], together with the detection of progestin binding sites by the dextran coated charcoal method [4] and by gel filtration [5], have confirmed and extended the existence of progesterone receptors in the hypothalamus and hypophysis [6–8]. The evidence for specific progesterone receptors in the hypothalamic and hypophysial nuclei is, however, still conflicting and unclear. While progestin uptake by brain cell nuclei has been reported autoradiographically in the guinea pig [6] and by liquid scintillation counting in the rat [9], others have failed to obtain conclusive evidence of saturable binding of progestin in cell nuclei in the rodent brain or pituitary [10–12].

In a preliminary report [3] we have detected a small but definite peak of [^{3}H]-R5020† binding in the 5S region on gradient patterns of 0.4 M KCl extract of purified nuclei from oestrogen-primed rat anterior hypophysial tissues incubated *in vitro* with [^{3}H]-R5020 at 37°C for 30 min, but very low amounts in the hypothalamic extract. Recently Milgrom *et al.*[13] and Walters and Clark[14] have reported that progesterone or R5020, a potent synthetic progestin, can exchange with progesterone bound in the uterine nuclei at low temperatures (0°C). In the present communication we have investigated progestin binding in nuclei from the hypothalamus and hypophysis of immature and mature female rats by examining an exchange reaction of R5020 or progesterone under various incubation conditions, by analysing saturable progestin binding, and by isolation of KCl-extractable progestin binding components by sucrose gradient centrifugation from purified nuclei and crude nuclear fractions.

Characterization of the progesterone cytosol receptors was also studied by exchange reaction at low temperature, the effect of high salt on the molecular changes, and separation on polyacrylamide agarose gel electrophoresis.

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† R5020: 17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione. R2858; 3,17β-dihydroxy-17α-ethinyl-11β-methoxy-estra-1,3,5(10)-triene. R1881; 17β-hydroxy-17α-methyl-estra-4,9,11-triene-3-one.

MATERIALS AND METHODS

Oestrogen priming

Oestradiol benzoate (5 or 10 μg) in sesame oil was injected subcutaneously (s.c.) into immature female rats 24 h before sacrifice at 28 days of age. Oestradiol valerate (2.5 mg) was injected as previously described [1], and 20 μg of oestradiol in sesame oil was injected s.c. 24 h before sacrifice at 87 days of age. In the experiments on nuclear progesterone receptors the oestrogen-primed immature and mature rats were s.c. injected with progesterone (1–5 mg) in sesame oil 1 h prior to sacrifice.

Dissection and cytosol preparation

The whole hypothalamus, including the preoptic nucleus, was cut out as a block as previously described [15]. The hypophyses and the anterior lobes of the pituitary were also collected from the immature and mature animals, respectively. The tissues were rinsed in ice-cold 10 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA and 12 mM thioglycerol (TET buffer), and were blotted on filter paper. The hypothalamic and hypophysial tissues were homogenized in 10% glycerol-TET buffer, and cytosol fractions (105,000 *g*) were obtained as previously described [1], unless otherwise stated.

Preparation of crude and purified nuclei

Crude nuclei (800 *g* *pellet*). The homogenates were centrifuged at 800 *g* for 20 min in Hitachi RP65 Model ultracentrifuge at 4°C. The 800 *g* pellet was suspended in 0.25 M sucrose, 10 mM Tris–HCl (pH 7.4), 12 mM thioglycerol, 2 mM $MgCl_2$ and 10 mM KCl (0.25 M sucrose-TMK buffer) and centrifuged at 800 *g* for 20 min. After washing the pellet three times with the same buffer, the washed pellet was suspended in 0.25 M sucrose-TMK buffer.

Purified nuclei. Hypothalami (1.42 g) and anterior hypophyses (0.88 g) were homogenized in 2.0 vol. (w/v) of 0.25 M sucrose-TMK as previously described [1]. The mixture was well mixed with 2.0 vol. (v/v) of 2.3 M sucrose-TMK solution, and then layered on the top of 2.3 M sucrose-TMK solution followed by centrifugation at 234,000 *g* for 27 min. The pellet was washed twice with 0.25 M sucrose-TMK buffer.

Incubation for binding and exchange of R5020 in nuclei

Effects of temperature and time. Immature female rats primed with oestradiol benzoate (10 μg) for 3 consecutive days were treated *in vivo* for 1 h with progesterone (5 mg). The nuclear pellet suspension (0.2 ml) from the hypothalami (DNA 56.8 μg) or hypophyses (DNA 18.4 μg) were incubated for varying times (0, 0.5, 1, 2, 4 and 8 h) at graded degrees (0, 10, 20 and 30°C) with 14.3 nM [^{3}H]-R5020 in the presence or absence of a 100-fold excess of unlabelled R5020. After the incubation the suspensions were centrifuged at 800 *g* for 20 min, and then the pellet was washed three times with 3 ml of the TET-10% glycerol buffer. The washed pellet was extracted with 3 ml of absolute ethanol overnight at room temperature. Aliquots of the ethanol extract (1 ml) was measured for radioactivity. The remaining nuclear pellet was assayed for DNA and protein.

Exchange of unlabelled R5020 for [^{3}H]-*R5020.* In order to confirm whether the binding of R5020 in the tissues is due to an exchange reaction, an additional experiment was carried out. Nuclei obtained from immature female rats injected s.c. with oestradiol (5 μg) and 24 h later with progesterone (1 mg) were washed three times with 0.25 M sucrose-TMK buffer and incubated at 10°C for 2 h with [^{3}H]-R5020 (12.7 nM). After being kept in ice for 15 min, 1.27 μM unlabelled R5020 was added to tubes previously incubated with [^{3}H]-R5020. Incubation was further continued at 0, 10 and 20°C for 0.5, 1, 2, 3 and 4 h, followed by centrifugation at 800 *g* for 20 min. The pellet was washed once with 0.5% Triton X-100–0.25 M sucrose-TMK buffer and twice with 10% glycerol–0.25 M sucrose-TMK buffer. The washed pellet was extracted with absolute ethanol and aliquots of the extract was counted.

Saturation analysis of nuclear binding. Hypothalamic and hypophysial nuclei were obtained from immature female rats treated *in vivo* for 24 h with oestradiol (5 μg) and for 1 h with progesterone (1 mg). The nuclei from hypothalami (DNA 56.8 μg) or hypophyses (DNA 18.4 μg) were incubated at 10°C for 2 h in the presence of varying concentrations of [^{3}H]-R5020 (0.76–24.3 nM) or [^{3}H]-R5020 plus a 100-fold excess of unlabelled R5020. Nuclear binding was plotted using the Scatchard plot [16].

Experiments on the isolation of progesterone binding by gradient centrifugation from purified and crude nuclei

Hypothalami and anterior hypophyses freshly dissected from the oestradiol valerate-primed female adult rats, which were treated *in vivo* for 1 h with progesterone (2 mg), were divided into two equal portions of 30 tissues from which the crude or purified nuclei was prepared.

For the exchange of progesterone bound in the nuclei the crude or purified nuclei from hypothalami and anterior hypophyses suspended in 0.21 ml of 0.25 M sucrose-TMK buffer were incubated at 10°C for 2 h with [^{3}H]-R5020 (12.7 nM). After centrifugation of the incubates at 800 *g* for 20 min at 4°C, the pellet was washed with 10 ml of 0.5% Triton X-100 and 10% glycerol–0.25 M-TMK buffer, and then centrifuged at 800 *g* for 20 min. This pellet was washed twice with 10 ml of 10% glycerol 0.25 M sucrose-TMK buffer, followed by centrifugation at 800 *g* for 20 min. The washed crude or purified nuclei from the hypothalami and anterior hypophyses were extracted with 0.4 M KCl –Tris–HCl buffer (pH 7.4) at 0°C for 60 min, and then centrifuged at 800 *g* for 20 min. The 0.4 M KCl extract from crude and purified hypothala-

mic nuclei were 1.15 and 0.82 ml, respectively. The extract from crude and purified hypophysial nuclei were 1.0 and 0.98 ml, respectively. Aliquots (0.6 ml) of the KCl extract were layered on the top of 5 ml 5–20% sucrose gradients containing 10% glycerol and 0.4 M KCl, and centrifuged at 234,000 *g* for 22 h. Aliquots (50 μl) of the KCl extract were measured for radioactivity and protein.

The pellet obtained from the nuclei after extraction with KCl, was further extracted with 3 ml ethanol, centrifugated at 800 *g* for 20 min and 1 ml of the 3 ml of supernatant was counted. The pellet was measured for DNA.

Progestin binding and exchange in cytosol

Cytosols from anterior hypophyses and hypothalami of oestrogen-primed adult female rats and from hypothalami from oestrogen-primed immature 28-day-old female rats were used. R5020 binding and exchange in the cytosols were carried out according to modified method of Walters and Clark [14].

Sucrose density gradient centrifugation

Centrifugation was carried out on 5–20% sucrose density gradients containing 10% glycerol in the presence or absence of 0.4 M KCl as previously described [1]. After sucrose density gradient centrifugation, the tubes were divided into 0.2 ml fractions with an ISCO Medol Density Gradient Fractionator and OD was measured at 260 nm. After the removal of excess unbound steroid in each fraction with Dextran-coated charcoal the radioactivity was counted as previously described [1]. Apparent sedimentation coefficient (S) was determined by the method of Martin and Ames[17]. Crystalline bovine serum albumin (4.6S), yeast alcohol dehydrogenase (7.6S), and beef liver catalase (11.3S) were used as standards.

Polyacrylamide agarose gel electrophoresis of cytosol steroids receptors

Polyacrylamide electrophoresis was performed in gels containing 3.25% acrylamide and 0.5% agarose as described by Naess *et al.*[18].

Hypothalamic cytosols from oestradiol benzoate-primed 28-day-old female rats (0.3 ml, 1.91–1.97 mg protein for [^{3}H]-R5020 and [^{3}H]-R1881; 2.28 mg for [^{3}H]-R2858) were incubated at 0°C for 18 h with [^{3}H]-R5020 (4.3 nM), [^{3}H]-R2528 (4.1 nM) and [^{3}H]-R1881 (3.7 nM), in the presence or absence of a 10-fold excess of respective steroids. After treatment with Dextran-coated charcoal for removal of excess unbound labelled steroids, an aliquot (0.25 ml) was applied on polyacrylamide agarose gel disc electrophoresis and run with 2 mA/tube for 156 min. Hypophysial cytosols (0.51–0.54 mg protein for [^{3}H]-R5020, 0.88–0.95 for [^{3}H]-R2858, 0.61–0.71 for [^{3}H]-R1881) were incubated with their respective [^{3}H]-steroids and the electrophoresis was carried out in the same fashion as the hypothalamus. The time of electrophoresis was 144 min.

Other procedures

Radioactivity was measured in a Triton–toluene–PPO–POPOP system with a counting efficiency of 38%. For analysis of binding kinetics by a Scatchard plot, specific R5020 binding was calculated as the difference in the radioactivity bound in the absence and presence of a 100-fold excess of unlabelled R5020. Protein was measured by the method of Lowry *et al.*[19]. DNA was measured by the method of Burton [20].

Chemicals

[6,7-^{3}H]-R5020 (S.A. 87.1 Ci/mmol) and radioinert R5020 were purchased from New England Nuclear Corp. [^{3}H]-R2858 (S.A. 52 Ci/mmol), [^{3}H]- R1881 (S.A. 58.2 Ci/mmol) and the respective unlabelled steroids were kindly supplied by Dr. J. P. Raynaud, Roussel Uclaf, Romainville, France. All [^{3}H]-steroids were purified by t.l.c. as previously described [15]. Other chemicals used in the experiments were identical to those previously described [1].

RESULTS

Nuclear progesterone receptors

Exchange of [^{3}H]-R5020 for nuclear bound progesterone. The characteristics of [^{3}H]-R5020 exchange for nuclear receptor sites was assessed in hypothalami and hypophyses from 28-day-old female rats primed with oestradiol (10 μg) for 3 consecutive days and injected with 5 mg progesterone 1 h before sacrifice. Nuclei (800 *g* pellet) were incubated with [^{3}H]-R5020 at 0, 10, 20 or 30°C for varying times in the presence or absence of unlabelled R5020. As shown in Fig. 1, exchange of [^{3}H]-R5020 in the hypothalamus at 0 and 10°C was complete after 2 h and remained stable for at least 2 h, followed by a slight decrease at 8 h. The exchange at 20°C decreased to three-quarters of that at 0°C. The degree of exchange markedly diminished with a rapid decrease with time 30°C.

The hypophysial binding reached a maximum by 1 h at all incubation temperatures tested and remained on a plateau for at least 3 h at 0 and 10°C (Fig. 1). The binding at 30°C rapidly decreased with time. It is noteworthy that less binding was observed in the hypophysis than the hypothalamus.

From the above data it is concluded that the binding/exchange of [^{3}H]-R5020 for progesterone bound in the nuclear receptors in the hypothalamus and hypophysis is achieved after 2 h at low temperature (0 and 10°C). Incubation at 10°C seems to provide a more suitable condition for the exchange reaction than at 0°C. In the following experiments the exchange condition at 10°C for 2 h was used, unless otherwise stated.

In order to confirm that this binding of [^{3}H]-R5020 at low temperature was due to an exchange reaction for unlabelled progesterone bound to nuclear receptors in these tissues, we carried out

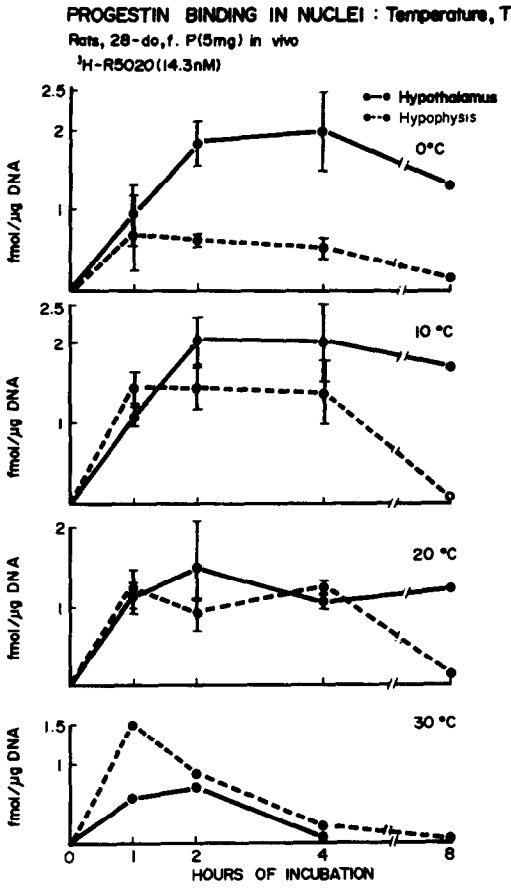

Fig. 1. R5020 binding in rat hypothalamic and hypophysial nuclei. Effects of temperature and time of *in vitro* incubation. Nuclei from oestradiol-primed (E_2 10 μg × 3 days) rats treated 1 h *in vivo* with progesterone (5 mg) were incubated for varying times at various degree with 14.3 nM [^{3}H]-R5020 and a 100-fold excess of R5020. —— Hypothalamus; --- hypophysis.

additional experiments on a re-exchange of unlabelled R5020 for [^{3}H]-R5020.

After specific [^{3}H]-R5020 binding was complete by incubation of hypothalamic and hypophysial nuclei at 10°C for 2 h, excess unlabelled R5020 (a 100-fold) was added to the incubation tubes previously incubated only with [^{3}H]-R5020 (10°C, 2 h) and further incubated at varying degrees (0, 10, 20°C) for varying times (0.5 1, 2, 3 and 4 h). In this experiment the nuclei exchanged with [^{3}H]-R5020 were washed with the buffer containing 0.5% Triton. As shown in Fig. 2, the exchange of R5020 for [^{3}H]-R5020 was complete after 2 h and remained stable for at least 2 h thereafter at 0° and 10°C. The exchange patterns of R5020 for [^{3}H]-R5020 bound to hypophysial nuclei were almost identical to those for the hypothalamus (Fig. 2). In contrast with the preceeding data, less exchange was found in the hypothalamus than the hypophysis.

Specific binding of [^{3}H]-R5020 in nuclei from hypothalami and hypophyses from rats treated *in vivo* with progesterone was thus confirmed to be due to an exchange reaction of [^{3}H]-R5020 for progesterone. It is also noted that the exchange of R5020 for [^{3}H]-R5020 bound to nuclear receptors, like the exchange of [^{3}H]-R5020 for progesterone, was rapid and reached a maximum after 2 h at low temperature.

Saturation analysis of [^{3}H]-*R5020 binding to nuclei.* As shown in Fig. 3A and B, there is specific binding of [^{3}H]-R5020 saturated at 6.4 nM in the hypothalamic nuclei and at 12.7 nM in the hypophysial nuclei. Less capacity for nuclear R5020 binding was found in the hypothalamus than the hypophysis.

Scatchard analysis of these specific binding curves yielded K_d = 1.4 nM and NBS = 466 fmol/mg DNA for the hypothalamus (Fig. 3A), and K_d = 6.4 nM and NBS = 915 fmol/mg DNA for the hypophysis (Fig. 3B).

Isolation of progesterone receptor protein complexes from crude (800 g pellet) and purified nuclei. In order to obtain further evidence for the nuclear progesterone receptors we have attempted to isolate the receptors labelled with [^{3}H]-R5020 at 10°C by gradient centrifugation from purified and crude nuclei from both hypothalami and anterior hypophyses.

The oestradiol valerate-primed adult female rats were s.c. injected with progesterone (2 mg) 1 h before sacrifice. The 800 *g* pellet and purified nuclei from hypothalami and anterior hypophyses were incubated at 10°C for 2 h with [^{3}H]-R5020 followed by extraction of nuclear binding components with 0.4 M KCl.

The sedimentation patterns of [^{3}H]-R5020-receptor complexes of 0.4 M KCl from purified and crude hypothalamic nuclei are shown in Fig. 4. A definite peak of [^{3}H]-R5020 was found in the 5–6S region on gradients of both nuclear extracts in terms of radioactivity and specific radioactivity. As shown in Fig. 5, a single peak of labelled R5020 was found in the 4–5S region on gradients of hypophysial nuclear extracts.

As it is clear from data in Table 1, the values per DNA for the purified hypothalamic and hypophysial nuclei are almost equal, although the value per nuclear protein for the hypophysis is about twice as much as the hypothalamus, suggesting the same magnitude of progestin binding sites in nuclei of both the hypothalamus and anterior hypophysis. It is also noteworthy that KCl-unextractable radioactivity in the hypothalamic and hypophysial nuclear extracts was found in a considerably large amount. Physiological significance of the KCl-unextractable components needs further investigation (Table 1).

Some characterisation of cytosol progesterone receptors

Progesterone binding and exchange in cytosol. The hypothalamic and anterior hypophysial cytosols from oestradiol valerate-primed adult female rats were first incubated with [^{3}H]-R5020 for 16 h at 0°C, and then unlabelled R5020 (100-fold) was added to the incubation tubes. Further incubation was continued at 0,

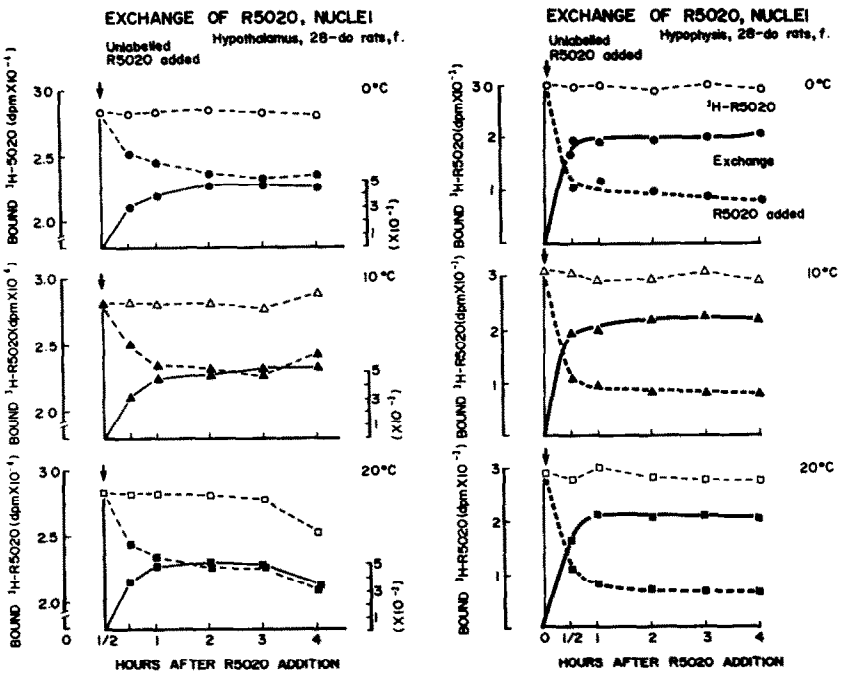

Fig. 2. Progesterone exchange in rat hypothalamic and hypophysial nuclei. After nuclei from hypothalami (56.8 μg DNA) or hypophyses (18.4 μg DNA) from oestradiol-primed 28-day-old rats treated 1 h *in vivo* with progesterone were incubated at 10°C for 2 h with 12.7 nM [^{3}H]-R5020, a secondary exchange of unlabelled R5020 for [^{3}H]-R5020 bound to the progesterone receptors was examined. 1.27 μM unlabelled R5020 was added to tubes previously incubated only with [^{3}H]-R5020. The incubation was performed at 0, 10 and 20°C for 0.5, 1, 2, 3 and 4 h.

--- with [^{3}H]-R5020 only (A); --- unlabelled R5020 added at 0 h (B); ——A–B.

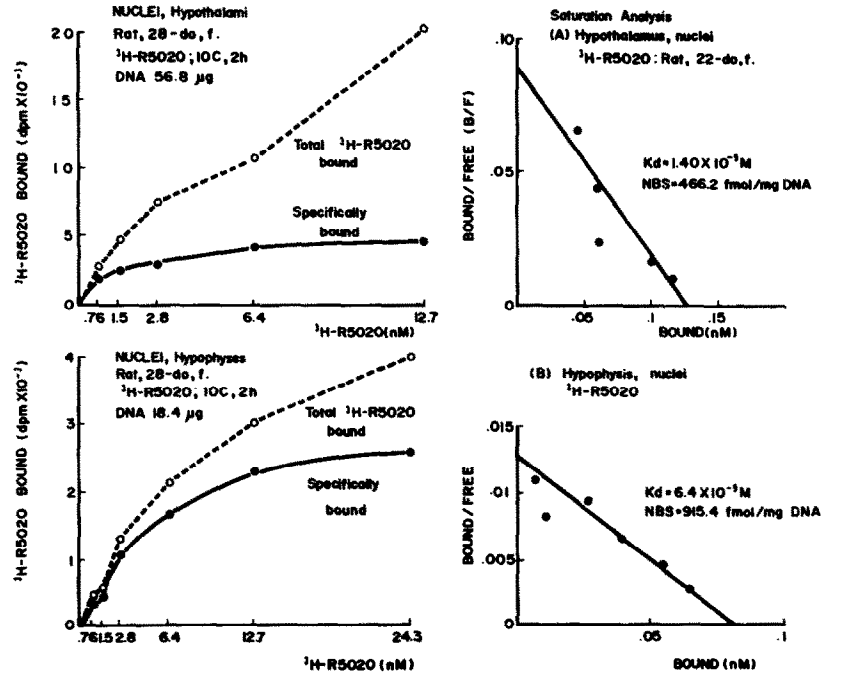

Fig. 3. Saturation analysis of nuclear progesterone receptors in the hypothalamus and hypophyses from the oestrogen primed 28-day-old female rats. Hypothalamic and hypophysial nuclei (800 *g*) was incubated at 10°C for 2 h in the presence of varying concentrations of [^{3}H]-R5020 or [^{3}H]-R5020 ± 100-fold R5020. Each animal was injected s.c. with 5 μg of oestradiol 24 h and 1 mg of progesterone 1 h prior to sacrifice. The amounts of specifically bound [^{3}H]-R5020 were plotted by the method of Scatchard [16]. (A) Hypothalamus; (B) hypophysis.

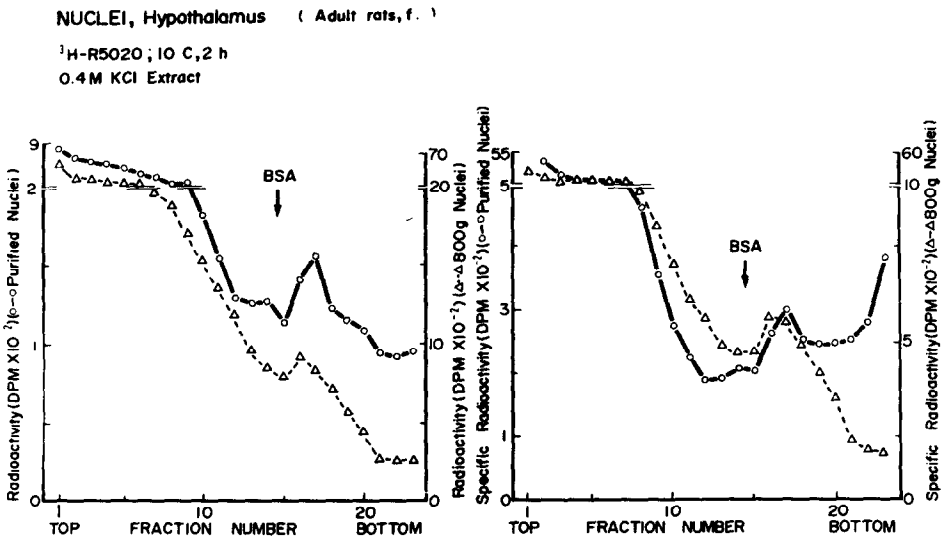

Fig. 4. Sucrose density gradients patterns of the 0.4 M KCl-extractable progesterone receptors from adult rat hypothalamic purified and crude nuclei incubated *in vitro* with [^{3}H]-R5020 at 10°C.

10, 20 or 30°C for varying times (0.5, 1, 2, 3, 4 and 8 h).

As shown in Fig. 6A, the exchange of unlabelled R5020 for [^{3}H]-R5020 bound to the cytosol receptors was complete by 2 h and remained stable for at least 2 h thereafter at 0, 10 or 20°C. At 30°C, however, the binding of [^{3}H]-R5020 and the exchange were markedly decreased, indicating possible disruption of cytosol receptors. As shown in Fig. 6B, similar results were obtained in the hypophysial cytosols. These indicate the exchange reaction of unlabelled R5020 for [^{3}H]-R5020 bound to the cytosol receptors.

Effect of KCl on progesterone cytosol receptors. Hypothalamic cytosols (3.13 mg/protein/0.3 ml) were incubated with [^{3}H]-R5020 (4.1 nM) for 4 h at 0°C and centrifuged on 5–20% sucrose density gradients containing 10% glycerol. Fractions (No. 11–16) corresponding to 7S peak, judged from standard enzymes (BSA and alcohol dehydrogenase), were collected and concentrated to 0.6 ml. The concentrated fractionate

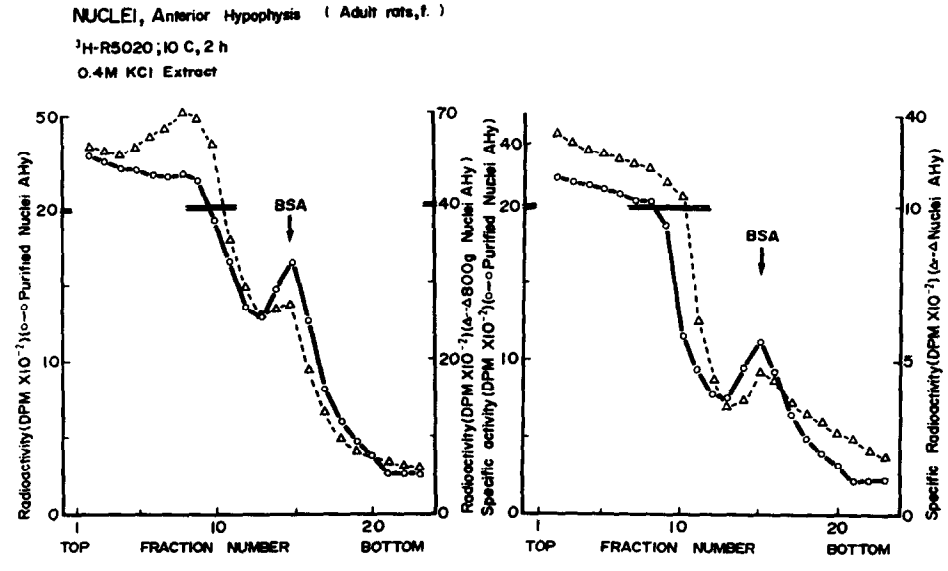

Fig. 5. Sucrose density gradients patterns of the 0.4 M KCl-extractable progestin receptors from adult rat anterior hypophysial purified and crude nuclei incubated *in vitro* with [^{3}H]-R5020 at 10°C. The detail of the experimental conditions was described in the text. O——O Purified nuclear extract; △---△ crude nuclear extract.

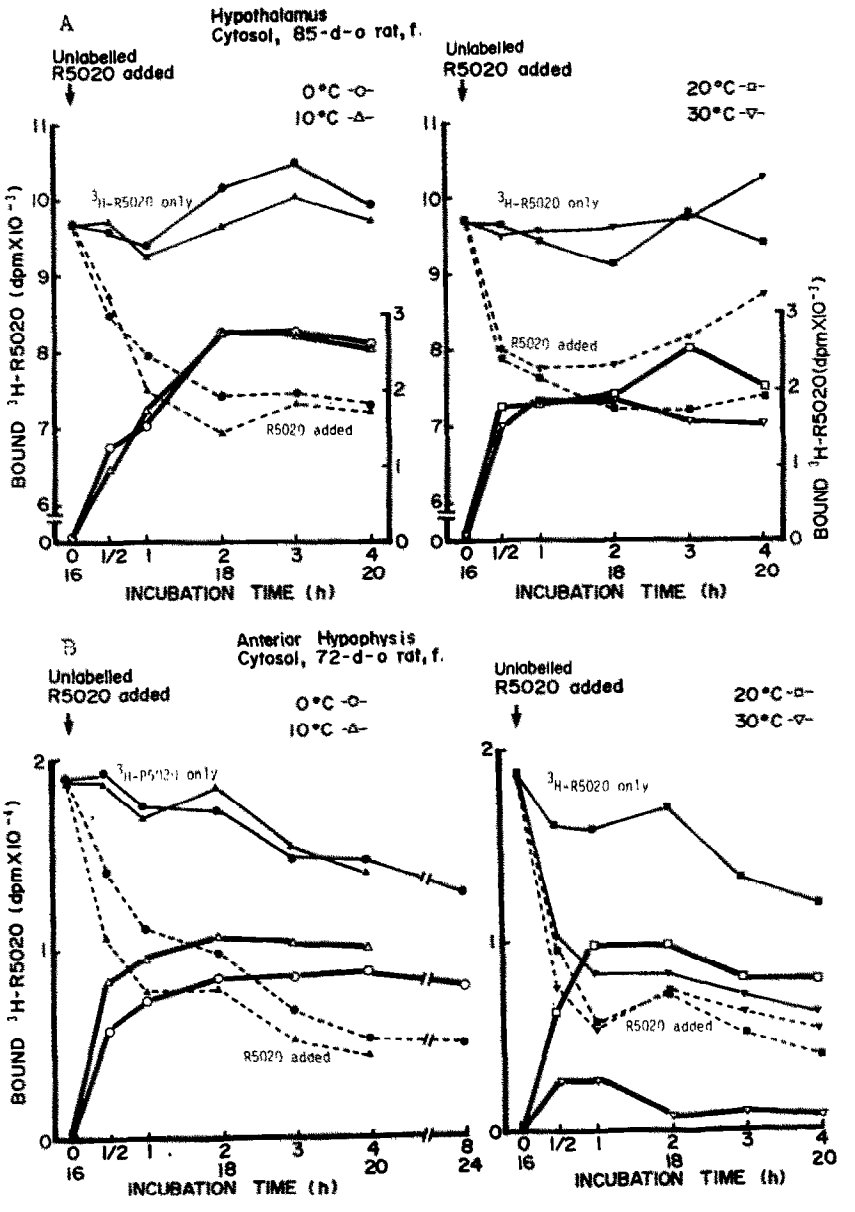

Fig. 6. Progesterone exchange in adult hypothalamic and anterior hypophysial cytosols. After 16 h incubation at 0°C in the presence of 10.7 nM [^{3}H]-R5020, tubes received 100-fold excess R5020 and the exchange phenomenon was measured. Cytosols from hypothalami (272 μg/0.25 ml) and hypophyses (256 μg/0.25 ml) were incubated at 0, 10, 20 or 30°C for varying times in the presence of 10.7 nM [^{3}H]-R5020 (S.A. 87.1 Ci/mmol) ± 100-fold excess R5020. (A) Hypothalami, (B) anterior hypophyses. Only [^{3}H]-R5020 (S.A. 87.1 Ci/mmol) ± 100-fold excess R5020. (A) Hypothalami, (B) anterior hypophyses. Only [^{3}H]-R5020: ●——● 0°C, ▲——▲ 10°C, ■——■ 20°C, ▼——▼ 30°C; unlabelled R5020 added: ●---● 0°C, ▲---▲ 10°C, ■---■ 20°C, ▼---▼ 30°C; amount of exchange of R5020 for labelled R5020: ○——○ 0°C, △——△ 10°C, □——□ 20°C, ▽——▽ 30°C.

was divided into an equal volume (0.3 ml each). Each fraction, containing 2.24 mg protein/0.3 ml, was further incubated in the presence of final concentration of 0.4 M KCl or its absence, followed by centrifugation at 50,000 *g* for 18 h in a SW 51 rotor in the presence or absence of 0.4 M KCl-sucrose density gradients containing 10% glycerol.

Anterior hypophysial cytosols (0.999 mg protein/0.3 ml) were incubated in the presence or absence of 0.4 M KCl under the same conditions as those for the hypothalamus. The mixture was centrifuged at 50,000 *g* for 18 h on sucrose gradients containing 10% glycerol with or without 0.4 M KCl.

As shown in Fig. 7, the 7S peak appearing in the controls without KCl treatment was found to be located in the 4S region after the KCl treatment.

Table 1. Radioactivity of KCl-extractable and unextractable fractions in purified and crude nuclei of adult female rat hypothalamus and anterior hypophysis.*

	Hypothalamus		Anterior hypophysis	
	Purified nuclei	800 *g* nuclei	Purified nuclei	800 *g* nuclei
KCl-extractable:†				
Total count (fmol)	45.2	406.8	414.3	859.3
Fmol/protein mg	93.2	199.6	211.4	312.3
Fmol/mg DNA	308.2	836.7	270.0	448.7
KCl-unextractable:				
Total count	169.9	3127.4	865.9	1904.1
Fmol/protein mg	350.3	1534.5	441.8	473.3
Fmol/mg DNA	1158.7	6431.7	564.3	1000.2

* Hypothalamic purified and crude nuclei were prepared from 1.415 and 1.323 g wet wt., respectively. Hypophysial purified and crude nuclei were obtained from 0.849 and 0.765 g wet wt., respectively. Purified and crude (800 *g*) nuclei were exchanged with [^{3}H]-R5020 at 10°C for 2 h.

† Radioactivity of the KCl-extractable fraction from nuclei exchanged with [^{3}H]-R5020 at 10°C for 2 h. Aliquots (0.05 ml) of the extract was measured directly for the radioactivity.

either in the hypothalamus or in the anterior hypophysis. This indicates that the 7S receptor is an aggregate form of the 4S receptors subunits.

Separation of cytosol progesterone receptors on polyacrylamide agarose gel electrophoresis. In order to investigate the characterization of progesterone receptors, the cytosols of rat hypothalamus and hypophysis incubated *in vitro* with [^{3}H]-R5020 at 0°C were analyzed on polyacrylamide agarose gel electrophoresis. Hypothalamic and hypophysial receptors for oestrogen and androgen labelled with [^{3}H]-R2858 [21] and [^{3}H]-R1881 [22], respectively, were also run for comparative purposes.

As shown in Fig. 8, the binding of [^{3}H]-R5020 was clearly separated from the oestrogen- and androgen-binding in the hypothalamic and hypophysial cytosols.

DISCUSSION

In the present experiments we have demonstrated specific progesterone receptor complexes in nuclei from the hypothalamus and hypophysis of immature and mature female rats on the basis of an exchange reaction of R5020 or progesterone at low temperature, saturable binding sites, and isolation of KCl-extractable progestin binding components by gradients centrifugation from purified and crude nuclei.

Progestin binding in the hypothalamic and hypophysial nuclei from female rats treated *in vivo* for 1 h

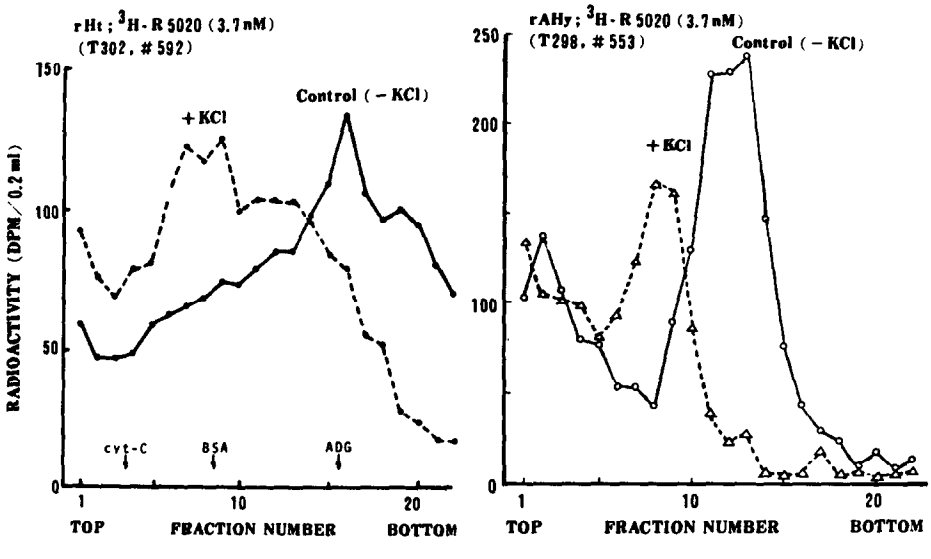

Fig. 7. Density gradient patterns of hypothalamic and anterior hypophysial progesterone receptors labelled with [^{3}H]-R5020. The 7S peak [^{3}H]-R5020 binding was collected and incubated *in vitro* with [^{3}H]-R5020 in a medium with or without high salt (0.4 M KCl). —— Control (without KCl); --- with KCl.

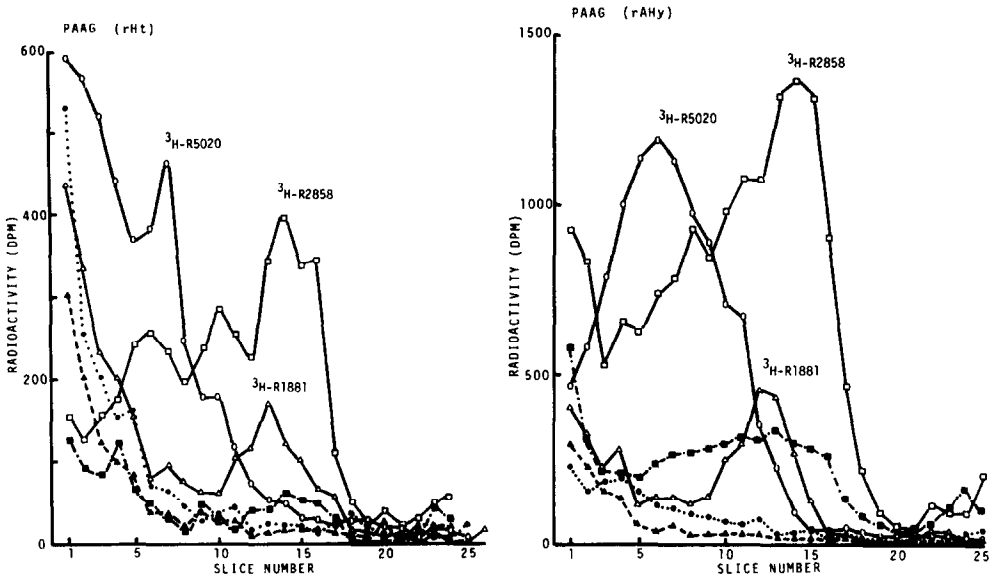

Fig. 8. Separation of receptors for progesterone, oestrogen and androgen in rat hypothalamic and hypophysial cytosols on polyacrylamide agarose gel. Electrophoresis of the cytosols labelled with [^{3}H]-R5020, [^{3}H]-R2858 and [^{3}H]-R1881 was carried out in gels containing 3.25% acrylamide and 0.5% agarose. ○——○ [^{3}H]-R5020; ●——● [^{3}H]-R5020 in the presence of unlabelled R5020; □——□ [^{3}H]-R2858; ■——■ [^{3}H]-R2858 in the presence of unlabelled R2858; △——△ [^{3}H]-R1881, ▲——▲ [^{3}H]-R1881 in the presence of unlabelled R1881.

with progesterone is rapid and completed by 2 h at 0 and 10°C but markedly diminished at 30°C (Fig. 1). This is in good agreement with the exchange of progesterone or R5020 in the uterus at 0°C [13, 14]. Furthermore, this progestin binding is found to be due to an exchange reaction as additional R5020 will exchange with [^{3}H]-R5020 bound to the hypothalamic and hypophysial nuclei (Fig. 2). In our previous reports [3], there existed only small amounts of [^{3}H]-R5020 binding on gradients of KCl extract of purified nuclei from rat hypothalamic and hypophyses incubated with [^{3}H]-R5020 at 37°C for 30 min. Some [^{3}H]-R5020 binding to the receptors may survive during the short term incubation even at high temperatures.

As clearly shown in Figs 4 and 5, progestin binding components extracted with 0.4 M KCl from the crude and purified nuclei labelled *in vitro* with [^{3}H]-R5020 at low temperatures (10°C) were identified on gradient profiles of adult female rat hypothalami and anterior hypophyses. These provide the direct evidence for the existence of nuclear progestin receptor complexes in the tissues.

A considerable amount of radioactivity was present in the KCl-unextractable nuclear fraction even from the purified hypothalamic and hypophysial nuclei (Table 1). In this context, it is interesting to mention the report of Pasqualini *et al.*[23] that oestrogen receptors resistant to extraction with 0.3–0.4 M KCl exist in fetal guinea pig brain. These resistant nuclear sites have also been found for oestradiol receptors in immature rat uterus [24] and in the kidney, lung and uterus of fetal guinea pigs [25], and for androgen receptors in rabbit epididymis [26]. The function and significance of the nuclear sites in these tissues are entirely unknown.

Nuclear progesterone receptors, together with the cytosol progesterone receptors in the hypothalamus and hypophysis, may provide further evidence for interaction of progesterone with the brain receptors as the basis of the mechanism of its central action.

The cytosol progesterone receptors in the hypophysis and hypothalamus, like the uterus [13], can exchange with R5020 or progesterone at low temperatures (Fig. 6). The cytosol receptor is heat-labile with rapid degradation at higher temperatures (30°C) especially in the hypophysis (Fig. 6). The progesterone receptor macromolecule proteins of 7S at low ionic strength seems to dissociate into 4S subunits, suggesting an aggregate-subunit relationship (Fig. 7). As clearly shown in Fig. 8, the progesterone receptor proteins in the hypothalamic and hypophysial cytosols are well separated on polyacrylamide agarose gels from oestrogen- and androgen-receptors, although they sedimented in the 7–8S region on gradients sedimentation. This suggests some differences in physico-chemical properties between these receptor proteins.

Acknowledgements—The authors thank Dr. J. P. Raynaud, Roussel-Uclaf, Romainville, France for supplying [^{3}H]-R2858 and [^{3}H]-R1881 and Profs. S. Okinaga and K. Arai for generous assistance. They also thank Miss M. Takamatsu for skillful assistance and Mrs. Y. Onda for typing the manuscript. Supported in part by grants from The Ministry of Education, Science and Culture.

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