J. Steroid Biochem. Molec. Biol. Vol. 38, No. 3, pp. 331–337, 1991
Printed in Great Britain. All rights reserved

0960-0760/91 $3.00 + 0.00
Copyright © 1991 Pergamon Press plc

19-HYDROXYANDROSTENEDIONE DOES NOT MODULATE [^{3}H]ALDOSTERONE BINDING TO HUMAN MONONUCLEAR LEUCOCYTES AND RAT RENAL CYTOSOL

HIROSHI MURASE,* KEIGO YASUDA, LEILANI B. MERCADO-ASIS, AKIHIRO MORI, TAKESHI SHIMADA, TOMOATSU MUNE, HIROYUKI MORITA, NOBUYASU NORITAKE, NORIYOSHI YAMAKITA and KIYOSHI MIURA

The Third Department of Internal Medicine, Gifu University School of Medicine, Tsukasa-machi 40, Gifu 500, Japan

(Received 6 August 1990)

Summary—To verify the aldosterone amplifying action of 19-hydroxyandrostenedione (19-OH-AD), we investigated [^{3}H]aldosterone and [^{3}H]19-OH-AD binding to type I (mineralocorticoid) receptor in the renal cytosol of adrenalectomized and ovariectomized rat, and human mononuclear leucocytes (MNL).

In the [^{3}H]aldosterone binding study, the cytosol was incubated with [^{3}H]aldosterone and 200-fold RU28362 (11β,17β-dihydroxy-6-methyl-,17α-(1-propynyl)-androsta-1,4,6-trien-3-one), a pure glucocorticoid, with or without 19-OH-AD. Scatchard plots of [^{3}H]aldosterone binding to cytosol with 0.2 or 20 nM 19-OH-AD or without 19-OH-AD were linear. Dissociation constants (K_d) and maximum bindings (B_{max}) without 19-OH-AD, and with 0.2 and 20 nM 19-OH-AD were: 0.71 $\pm$ 0.03 nM and 23.0 $\pm$ 3.4 fmol/mg protein (mean $\pm$ SD, $n = 3$), 0.72 $\pm$ 0.05 nM and 23.1 $\pm$ 2.3 fmol/mg protein ($n = 3$), and 0.77 $\pm$ 0.04 nM and 22.9 $\pm$ 4.8 fmol/mg protein ($n = 3$), respectively. 19-OH-AD did not significantly change the K_d and B_{max} of [^{3}H]aldosterone binding. A high concentration of 19-OH-AD slightly displaced 0.2 or 5 nM [^{3}H]aldosterone bound to cytosol. In human MNL, Scatchard plots of [^{3}H]aldosterone binding with both 0.2 and 20 nM 19-OH-AD and without 19-OH-AD were linear. K_d and B_{max} were, respectively, 1.00 nM and 780 sites/cell in the absence of 19-OH-AD, and 1.07 nM and 774 sites/cell in the presence of 0.2 nM 19-OH-AD. Without 19-OH-AD they were, respectively, 0.95 nM and 551 sites/cell, and 1.10 nM and 560 sites/cell with 20 nM 19-OH-AD. A high concentration of 19-OH-AD slighly displaced 0.2 or 5 nM of [^{3}H]aldosterone bound to MNL.

In both tissues, there was no obvious specific binding of [^{3}H]19-OH-AD within the range of 1–60 nM.

The above results suggest that the amplifying effect of 19-OH-AD on aldosterone mineralocorticoid action may not occur at the binding site of aldosterone to type I receptor, and that 19-OH-AD itself may not have any direct or indirect mineralocorticoid actions on the steroid receptor-mediated process in the rat kidney and human MNL.

INTRODUCTION

Sekihara and coworkers reported that 19-hydroxyandrostenedione (19-OH-AD) does not have intrinsic mineralocorticoid activity, but merely acts as an amplifier of the mineralocorticoid action of aldosterone in the adrenalectomized rat [1]. They speculated that such amplification is mediated via an enhancement of aldosterone binding to renal cytosol receptors. In man, elevated levels of plasma 19-OH-AD have been observed in normal- and low-renin hypertension [2] and in the hypertension of pregnancy [3], which suggest a pathophysiologic role for 19-OH-AD in such disease entities. On the other hand, recent studies have cast doubt on the aldosterone potentiating action of 19-OH-AD, and suggested that 19-OH-AD itself has a mild hypertensive action [4–6]. However, no evidence has yet been produced demonstrating the exact mechanism of the amplifying effect of 19-OH-AD on aldosterone, or of its own hypertensinogenic action. It remains to be resolved whether this action involves stimulation of the aldosterone binding to type I receptor, or a direct binding to specific or nonspecific steroid receptors.

*To whom correspondence should be addressed.

Armanini and colleagues have demonstrated that human mononuclear leucocytes (MNL) have specific binding sites for aldosterone in association with changes in intracellular sodium and potassium, and that MNL is a useful model for the study of type I receptor [7, 8]. In order to clarify the mechanism of the amplifying effect of 19-OH-AD on aldosterone at the receptor level, we investigated its effect on aldosterone binding to type I receptor, and studied its own binding to such receptors, using rat renal cytosol and human MNL.

MATERIALS AND METHODS

The following materials were purchased: [1,2-^{3}H]aldosterone ([^{3}H]aldosterone, 56 Ci/mmol) and [6,7-^{3}H]19-hydroxyandrostenedione ([^{3}H]19-OH-AD, 46.3 Ci/mmol) from New England Nuclear (Boston, Mass), 19-OH-AD and aldosterone from Sigma (St Louis, Mo.), RPMI-1640 with L-glutamine and 25 mM HEPES from M.A. Products (Walkersville, Mich.), Percoll from Pharmacia (Uppsala, Sweden), and a liquid scintillator, ACS II, from Amersham (Arlington Heights, Ill.). RU28362 (11β,17β-dihydroxy-6-methyl-17α-(1-propynyl)-androsta-1,4,6-trien-3-one), a pure synthetic glucocorticoid, was generously donated by Roussel–Uclaf (Romainville, France). Other chemicals were from Sigma (St Louis, Mo.). Female Sprague–Dawley rats were obtained from Chyubu Kagaku Shizai (Nagoya, Japan).

Preparation of rat renal cytosol

Cytosol was prepared by the method of Krozowski and Funder [9]. Female Sprague–Dawley rats (150–200 g) were adrenalectomized and ovariectomized 7 days before killing. The rats were maintained on 0.9% saline and standard laboratory diet *ad libitum*. Before nephrectomy, the kidneys were perfused with 50 ml of an ice-cold isotonic solution (0.9% NaCl, 10 mM Tris, pH 7.4) via a catheter placed into the abdominal aorta under pentobarbital anesthesia. The excised kidneys were minced and rinsed with an isotonic solution and then homogenized in TSMD buffer (100 mM Tris, 250 mM sucrose, 100 mM Na_2MoO_4, 2 mM dithiothreitol, pH 7.4). Homogenates were centrifuged at 105,000 g for 60 min at 4°C. Supernatants were stored at -80°C. The concentration of cytosol protein was determined by the method of Bradford [10] using bovine serum albumin as the standard.

Cytosol binding study

Aliquots of 300 μl of cytosol (1–3 mg protein/ml) were incubated for 18 h at 4°C with the various concentrations of [^{3}H]aldosterone with or without 19-OH-AD, and with 200-fold excess RU28362, type II (glucocorticoid) receptor specific agonist, to prevent the binding of the tracer to type II receptor [11]. The same aliquots were incubated for 18 h at 4°C with the increasing concentrations (1–40 nM) of [^{3}H]19-OH-AD. Receptor-bound steroid were separated from the cytosol with 300 μl of an ice-cold suspension of hydroxylapatite (15%, w/v) in 50 mM Tris, 10 mM KH_2PO_4, pH 7.2. After incubation for 30 min at 4°C, the tubes were centrifuged at 1000 g for 3 min at 4°C, the supernatants decanted, and the pellets washed three times with 1 ml of washing buffer (10 mM Tris, 5 mM NaH_2PO_4, 1.5 mM EDTA, pH 7.2 1% Tween 80). The washed hydroxylapatite pellets were resuspended in 2 ml of ethanol at room temperature for 60 min and centrifuged at 1000 g for 5 min at 4°C. The supernatants were transferred into counting vials and 10 ml ACS II added. The radioactivity was measured with a liquid scintillation counter (PR-2650, Packard, Downers Grove, Ill.).

Isolation of MNL

MNL were obtained with a Percoll density gradient following the method of Hjorth *et al.* [12] with Funder's modification (personal communication). Heparinized blood was obtained from fasting normal male volunteers after resting in the supine position for 60 min from 0800–0900 h. Percoll was made isotonic with 0.9% NaCl and was diluted with phosphate-buffered saline (PBS; 0.15 mM NaCl, 6.7 mM phosphate, pH 7.2) to 55% Percoll (final pH 7.4). 16 ml of heparinized blood was gently layered over the same volume of 55% Percoll in the centrifuge tubes. The tubes were centrifuged at 400 g for 30 min. The layer of MNL was harvested, the cells washed twice with saline, and the solution let stand for 10 min before each centrifugation to remove endogenous steroids and steroid-binding proteins [7]. Finally, the cell pellet was suspended in RPMI-1640. The number of cells was determined with a Bürker–Türk counting chamber. Polynuclear leucocytes constituted fewer than 5% of the cells as determined by the May–Giemsa double-staining method. The viability of the cells was 95–98% before and after incubation as judged

by the trypan blue exclusion method. The entire procedure was performed at 16–18°C.

MNL binding study

The procedure was performed using the method of Armanini *et al.* [7]. Aliquots of 500 μl of cell suspension (3–6 × 10^6 cells/tube) were incubated with the various concentrations of [^{3}H]aldosterone and 200-fold RU28362, with or without 19-OH-AD at 37°C for 60 min with constant shaking (110–120/min) under 5% CO_2 and 95% O_2. The same aliquots were incubated with the increasing concentrations (3–66 nM) of [^{3}H]19-OH-AD with or without 1000-fold 19-OH-AD for 60 min at 37°C. The reaction was stopped with 2 ml ice-cold PBS. The tubes were centrifuged at 750 *g* for 5 min at 4°C, and the pellets were washed twice with PBS. The pellets were resuspended in 1 ml of ethanol and let stand for 60 min at room temperature. Finally, the tubes were centrifuged at 2000 *g* for 5 min at 4°C, the supernatants transferred into counting vials, 10 ml ACS II added, and the radioactivity counted. Loss of MNL during the washing procedure after incubation was below 5%.

Specific binding in rat renal cytosol and human MNL was measured by subtracting non-specific binding from total binding. Nonspecific binding was obtained by incorporation of 1000-fold unlabeled aldosterone into the incubated mixture. Linear regression was applied in Scatchard analysis [13].

All data were expressed as the mean or mean ± SD. Statistical analysis was performed using unpaired Student's *t*-test. Statistical significance was defined as $P < 0.05$.

RESULTS

Cytosol binding study

Figure 1 shows Scatchard analysis of [^{3}H]-aldosterone binding in the presence or absence of 0.2 or 20 nM 19-OH-AD. The former concentration (0.2 nM) was almost equivalent to the plasma concentration used to induce hypertension in the rat experiment performed by Sekihara, in which 1 μg of 19-OH-AD was administered once a week subcutaneously [14]. Scatchard plots of [^{3}H]aldosterone binding were rectilinear both in the presence and absence of 19-OH-AD. The dissociation constant of binding (K_d) was 0.71 ± 0.03 nM ($n = 3$) and maximum binding (B_{max}) was 23.0 ± 3.4 fmol/mg protein ($n = 3$) without 19-OH-AD. In the presence of 0.2 or 20 nM 19-OH-AD, K_d and B_{max} were, respectively, 0.72 ± 0.05 nM and 23.1 ± 2.3 fmol/mg protein ($n = 3$) in the former, and 0.77 ± 0.04 nM and 22.9 ± 4.8 fmol/mg protein ($n = 3$) in the latter. Addition of low or high concentrations of 19-OH-AD had no significant effects on the binding parameters of aldosterone to type I receptor.

Unlabeled 100-fold aldosterone completely displaced 0.2 nM (physiological plasma concentration in rats [14]) and 5 nM of [^{3}H]aldosterone bound to type I receptor of rat renal cytosol (Fig. 2). 19-OH-AD, however, slightly displaced [^{3}H]aldosterone binding only when present at an extremely high concentration.

MNL binding study

The specific binding of [^{3}H]aldosterone reached a plateau at 60 min, and significantly correlated with the number of cells in the range of 4–12 × 10^6 cells/ml (data not shown). The specific binding of [^{3}H]aldosterone was saturable, and Scatchard plots of the binding were rectilinear (Fig. 3A and B). K_d and B_{max} were 1.21 ± 0.4 nM and 635 ± 172 sites/cell, respectively ($n = 13$).

Figure 4A and B show the effect of 19-OH-AD on [^{3}H]aldosterone binding in human

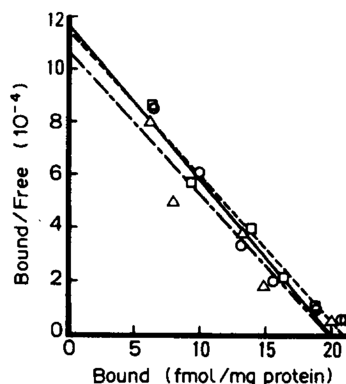

Fig. 1. Scatchard plots of [^{3}H]aldosterone binding to rat renal cytosol incubated without 19-hydroxyandrostenedione (19-OH-AD) (○), or with 0.2 (□) or 20 nM (△) 19-OH-AD. 300 μl of cytosol (1 mg protein/ml) was incubated with increasing concentrations of [^{3}H]aldosterone (0.3–12 nM) and 200-fold RU28362(11β,17β-dihydroxy-6-methyl,17α-(1-propynyl)-androsta-1,4,6,trien-3-one) at 4°C for 18 h and without 19-OH-AD or with 0.2 and 20 nM 19-OH-AD, with or without 1000-fold aldosterone (to measure nonspecific binding). Plots were obtained from a typical experiment. The dissociation constant (K_d) and the maximum binding (B_{max}) were 0.70 nM and 20.6 fmol/mg protein (○, $\gamma = -0.984$), and 0.74 nM and 21.1 fmol/mg protein (□, $\gamma = -0.984$), and 0.77 nM and 19.3 fmol/mg protein (△, $\gamma = -0.954$), respectively.

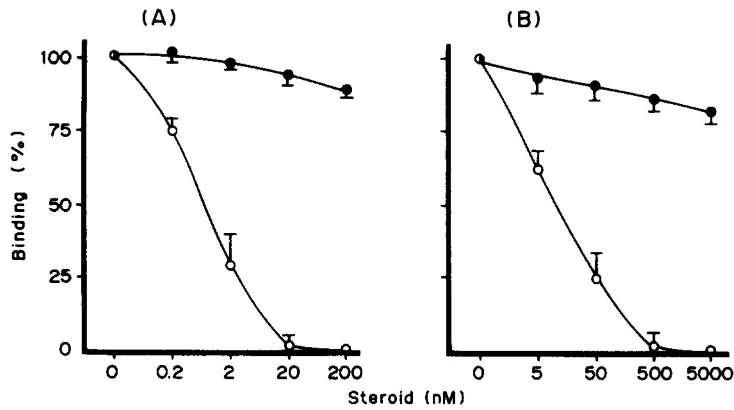

Fig. 2. Displacement study of 0.2 nM (A) and 5 nM (B) [^{3}H]aldosterone binding to rat renal cytosol by aldosterone (○) or 19-OH-AD (●). 300 μl of cytosol (2 mg protein/ml in A and 1 mg protein/ml in B) were incubated with 0.2 nM [^{3}H]aldosterone and 40 nM RU28362 with unlabeled steroids at 4°C for 18 h. For specific binding, the binding in the presence of 200 nM unlabeled aldosterone was subtracted from the total binding. Binding was represented as a percentage for the specific binding without authentic steroids. Plots were means ± SD of three experiments.

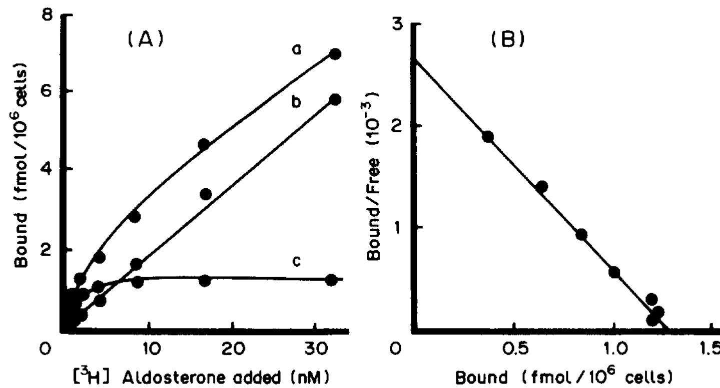

Fig. 3. Saturation curve of [^{3}H]aldosterone binding to human mononuclear leucocytes (MNL) (A) and Scatchard plot (B). Aliquots of 500 μl of MNL suspension (7.9 × 10^6 cells/ml) were incubated with increasing concentrations of [^{3}H]aldosterone (0.3–6 nM) and 200-fold RU28362 at 37°C for 60 min with or without 1000-fold aldosterone (to measure nonspecific binding). Plots were means of triplicate determinations in a typical experiment. K_d was 0.96 nM and B_{max} was 780 sites/cell ($\gamma = -0.99$). (A, line *a*; total binding, line *b*; nonspecific binding, line *c*; specific binding).

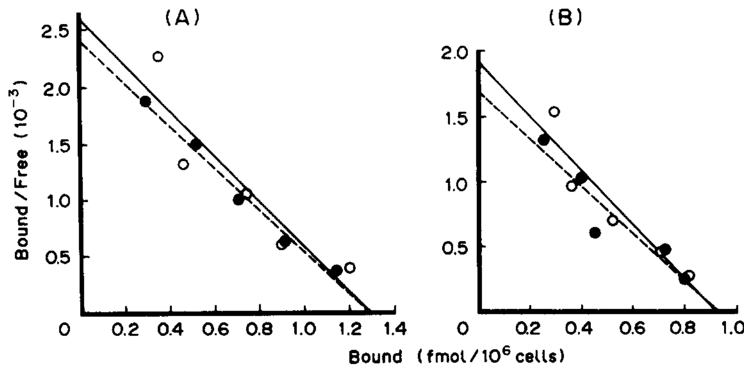

Fig. 4. Scatchard plots of [^{3}H]aldosterone binding to human MNL incubated without (○) or with 0.2 nM (●) 19-OH-AD (*panel* A) and without (○) or with 20 nM (●) 19-OH-AD (*panel* B). Aliquots of 500 μl of MNL suspension (5.4 × 10^6 cells/ml) in *panel* A and 7.4 × 10^6 cells/ml in *panel* B) were incubated with increasing concentrations of [^{3}H]aldosterone (0.3–6 nM) and 200-fold RU28362 at 37°C for 60 min without, or with 0.2 nM (A) or 20 nM (B) 19-OH-AD and with or without 1000-fold aldosterone. The results in Fig. 4A and B were obtained from different subjects. Plots were means of triplicate determinations. In pannel A, K_d and V_{max} were 1.0 nM and 780 sites/cell without 19-OH-AD (○, $\gamma = -0.92$), and 1.1 nM and 774 sites/cell with 0.2 nM 19-OH-AD (●, $\gamma = -0.99$). In *panel* B, K_d and B_{max} were 0.95 nM and 551 sites/cell without 19-OH-AD (○, $\gamma = -0.94$), and 1.1 nM and 560 sites/cell with 20 nM 19-OH-AD (●, $\gamma = -0.94$).

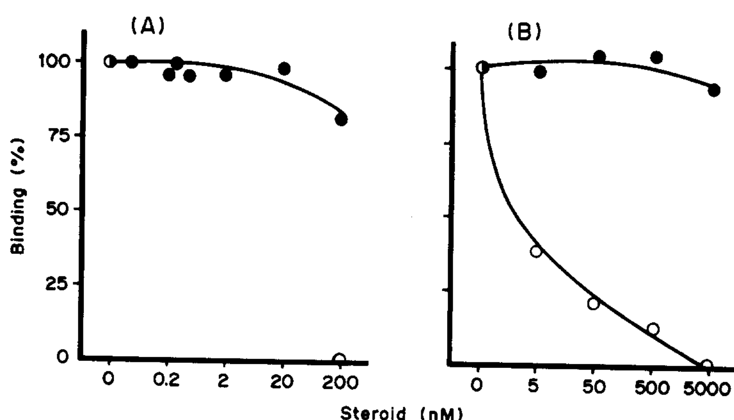

Fig. 5. Displacement of 0.2 nM (*panel* A) and 5 nM (*panel* B) [^{3}H]aldosterone binding to human MNL by unlabeled aldosterone (○) or 19-OH-AD (●). MNL suspensions (8.7 x 10^6 cells/ml in *panel* A and 11.3 x 10^6 cells/ml in *panel* B) was incubated with 0.2 nM [^{3}H]aldosterone and 40 nM RU28362 with unlabeled steroids at 37°C for 60 min. For specific binding, the binding in the presence of 200 nM unlabeled aldosterone was subtracted from the total binding. Binding was represented as a percentage for the specific binding without authentic steroids. Plots were means of triplicate determinations.

MNL. Scatchard plots of [^{3}H]aldosterone binding were rectilinear with or without 0.2 nM 19-OH-AD (the basal plasma concentration in man as measured by the method established in our laboratory) (Fig. 4A). K_d and B_{max} were, respectively, 1.0 nM and 780 sites/cell, without 19-OH-AD, and 1.1 nM and 774 sites/cell, with 0.2 nM 19-OH-AD. The addition of 19-OH-AD did not change either K_d or B_{max}. Scatchard plots of [^{3}H]aldosterone binding with or without 20 nM 19-OH-AD were also rectilinear (Fig. 4B). K_d and B_{max} were 0.95 nM and 551 sites/cell, respectively, without 19-OH-AD, and 1.1 nM and 560 sites/cell, respectively, with 20 nM 19-OH-AD. 19-OH-AD produced almost no change in B_{max}, while in the presence of 20 nM 19-OH-AD, K_d was slightly higher than in its absence.

In the displacement study by 19-OH-AD on 0.2 nM and 5 nM [^{3}H]aldosterone binding, 19-OH-AD even slightly inhibited the binding of [^{3}H]aldosterone to MNL when present at an extremely high concentration (Fig. 5).

[^{3}H]19-OH-AD binding study

As shown in Fig. 6, in both rat renal cytosol and human MNL, the binding of [^{3}H]19-OH-AD in the presence and absence of 19-OH-AD was almost equal. There was no specific binding of [^{3}H]19-OH-AD.

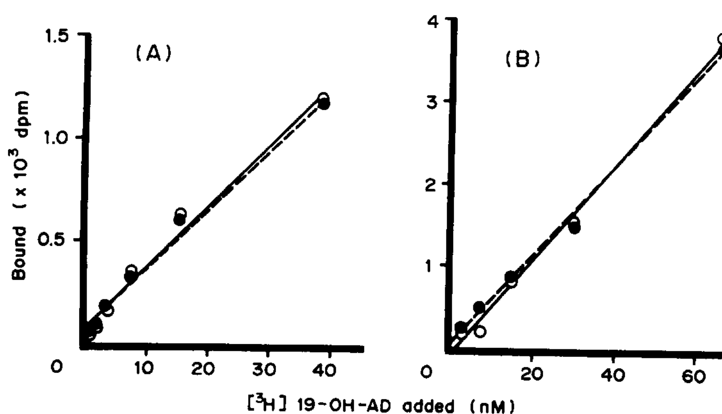

Fig. 6. The binding study of [^{3}H]19-OH-AD to rat renal cytosol (*panel* A) and human MNL (*panel* B). In *panel* A, the cytosol was incubated for 18 h at 0°C with increasing concentrations (1–40 nM) of [^{3}H]19-OH-AD, without (○——○; $\gamma = 0.992$) or with (●---●; $\gamma = 0.996$) 1000-fold 19-OH-AD. In *panel* B, human MNL was incubated for 60 min at 37°C with 3–66 nM [^{3}H]19-OH-AD, without (○——○; $\gamma = 0.997$) or with (●---●: $\gamma = 0.996$) 1000-fold 19-OH-AD. Plots were means of triplicate determinations in a typical experiment.

DISCUSSION

Since the first report on the amplifying effect of 19-OH-AD on aldosterone, Sekihara and colleagues have confirmed this effect and further elucidated the 19-OH-AD secretory mechanism and its etiologic role in hypertension [2, 14, 15]. Their results have suggested the possibility that 19-OH-AD is an unusual steroid which acts on an amplifying mechanism only when present at very low plasma concentrations and with its plasma level and that of aldosterone showing reciprocal changes, and secondly, on a secretory mechanism controlled by both ACTH and angiotensin-II regardless of C19 steroids [15]. In contrast, although Gomez-Sanchez and Gomez-Sanchez observed a mild hypertensinogenic effect of 19-OH-AD in rats, no enhancement of the mineralocorticoid properties of endogenous and exogenous aldosterone, or the previously reported remarkable hypertensinogenic properties in the rat were found [5]. On the other hand, studies on the mechanism of such amplifying or hypertensinogenic actions of 19-OH-AD have yet to be performed. In this regard, we assumed that these postulated actions of 19-OH-AD could be at the mineralocorticoid (type I) receptor level. Recently, the development of a synthetic compound, type II receptor-specific agonist RU28362 (Roussel–Uclaf), has facilitated these investigations.

Our results on the K_d and B_{max} of aldosterone binding in rat renal cytosol (the target organ of aldosterone) were similar to those of Sheppard and Funder [16]. Conversely, K_d and B_{max} in MNL, the application of which was developed for the study of aldosterone receptors in man by Armanini *et al.* [7], were slightly lower and slightly higher, respectively, than the values reported by Armanini *et al.* [7]. This may be explained by a difference in the property of the specific glucocorticoid analogue used. Armanini and his group [7] used RU26988 instead of the RU28362 used in the present study. The latter has a lower affinity for type I receptor and higher affinity for type II receptor [11].

In the present studies, specific binding of [^{3}H]aldosterone, as well as its K_d and binding capacity, was not modulated by the addition of 19-OH-AD in either the rat renal cytosol or human MNL experiments. This was observed in the case of the concentration of 19-OH-AD used by Sekihara to demonstrate its amplifying effects on sodium retention and hypertension [14] and also at a much higher concentration. These results indicate that the site of the amplifying action of 19-OH-AD on aldosterone mineralocorticoid action is not, at least, at the stage of aldosterone binding to type I receptor. Furthermore, there was no specific binding of [^{3}H]-19-OH-AD to the rat renal cytosol or human MNL within the range of concentrations used (1–60 nM). These findings suggest that 19-OH-AD does not have any direct or indirect action on the receptor-mediated process in the rat kidney. However, the present result is not an argument against the potentiating action of 19-OH-AD on aldosterone. In fact, the first steroid recognized as an amplifier of mineralocorticoid, 5α-dihydrocortisol, also showed its action without enhancement of aldosterone binding [17]. For the resolution of the unknown process involved in the potentiation of aldosterone action by 19-OH-AD, further studies on this potentiation are necessary.

Manabe *et al.* [4] and Gomez-Sanchez and Gomez-Sanchez [5] reported that 19-OH-AD itself has a hypertensinogenic effect but no amplifying effect on aldosterone. Mikami *et al.* reported that 19-OH-AD and aldosterone have a direct vasoconstrictive effect, which is not inhibited by spironolactone, on the *in vivo* rabbit ear artery [6]. The hypertensinogenic action of 19-OH-AD may be due to a direct vascular effect. In our study, no specific binding of 19-OH-AD to rat renal cytosol and human MNL was demonstrated, suggesting that the hypertensinogenic action of 19-OH-AD is not linked to steroid receptors.

Acknowledgements—This study was supported in part by research grants from the Ministry of Health and Welfare, Japan: The research Committees of "Disorders of Adrenal Hormones", and "Disorders of Hypothalamo-pituitary glands". We are grateful to Professor John W. Funder (Medical Research Center, Prince Henry's Hospital, Melbourne, Australia) for his kind advice on the method of aldosterone binding to mononuclear leucocytes. We also express our gratitude to Roussel–Uclaf (France) for the gift of RU28362.

REFERENCES

1. Sekihara H., Ohsawa N. and Kosaka R.: Amplification of the action of subthreshold doses of aldosterone by 19-hydroxyandrost-4-ene-3,17-dione. *Biochem. Biophys. Res. Commun.* **87** (1979) 827–835.
2. Sekihara H.: 19-hydroxyandrostenedione: a potent hypertensinogenic steroid in man. *J. Steroid Biochem.* **19** (1983) 353–358.
3. Martin J. D., Hahnel M. E. and Hahnel R.: 19-hydroxyandrostenedione- a factor in pregnancy hypertension. *Clin. Exp. Hyper.* **B4** (1985) 127–139.
4. Manabe R., Honda M. and Hatano M.: A comparative study of C19 steroid-induced hypertension and deoxycorticosterone acetate(DOCA)-salt hypertension. *Folia Endocr. (Jap.)* **60** (1984) 1328–1337.

5. Gomez-Sanchez E. P. and Gomez-Sanchez C. E.: A reevaluation of the mineralocorticoid and hypertensinogenic potential of 19-hydroxyandrostenedione. *Endocrinology* **118** (1986) 2582–2587.
6. Mikami H., Ogihara T. Ohde H., Katahira K., Kohara K. and Kumahara Y.: Direct vascular effects of 19-hydroxyandrostenedione. *Meth. Find. Expl. Clin. Pharmac.* **11** (1989) 241–248.
7. Armanini D., Strasser T. and Weber P. C.: Characterization of aldosterone binding sites in circulatory human mononuclear leucocytes. *Am. J. Physiol.* **248** (1985) E388–E390.
8. Wheling M., Armanini D., Strasser T. and Weber P. C.: Effect of aldosterone on sodium and potassium concentrations in human mononuclear leucocytes. *Am. J. Physiol.* **252** (1987) E505–E508.
9. Krozowski Z. S. and Funder J. F.: Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc. Natn Acad. Sci. U.S.A.* **80** (1983) 6056–6060.
10. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
11. Philibert D. and Moguilewsky M.: RU28362, a useful tool for the characterization of glucocorticoid and mineralocorticoid receptors. *Prog. of the 65th Ann. Mtg of the American Endocrine Society*, San Antonio, Texas (1983) p. 335. (Abstr.).
12. Hjorth R., Jonsson A-K. and Vretblat P.: A rapid method for purification of human granulocytes using Percoll. *J. Immun. Meth.* **43** (1981) 96–101.
13. Scatchard G.: The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51** (1949) 660–672.
14. Sekihara H.: 19-hydroxyandrostenedione; evidence for a new class of sodium-retaining and hypertensionogenic steroids. *Endocrinology* **113** (1983) 1141–1148.
15. Sekihara H., Torii R., Osawa Y. and Takaku F.: Angiotensin II induces the release of 19-hydroxyandrostenedione in man. *J. Clin. Endocr. Metab.* **61** (1985) 291–296.
16. Sheppard K. E. and Funder J. W.: Equivalent affinity of aldosterone and corticosterone for type I receptors in kidney and hippocampus: direct binding studies. *J. Steroid Biochem.* **28** (1987) 737–742.
17. Adam W. R., Funder J. W., Mercer J. and Ulick S.: Amplification of the action of aldosterone by 5α-dihydrocortisol. *Endocrinology* **103** (1978) 465–471.