Corticosteroid Binding in Human Artery

HIRONOBU SASANO, NORIMICHI WATANABE* and NOBUAKI SASANO†

Department of Pathology, Tohoku University School of Medicine, Sendai 980 and *Suifu Hospital, Mito 310

SASANO, H., WATANABE N. and SASANO, N. *Corticosteroid Binding in Human Artery*. Tohoku J. exp. Med., 1986, **150** (2), 117-125 — Mineralo- and glucocorticoid binding in cytosol of surgically obtained human arteries and umbilical arteries were analyzed. Scatchard analysis of glucocorticoid binding in femoral and umbilical arteries revealed the binding parameters of $K_d = 4.3, 8.2 \times 10^{-9}$ M; $B_{max} = 42, 90$ fmol/mg protein, respectively. Scatchard analysis of mineralocorticoid binding in inferior mesenteric artery showed a curvilinear pattern suggestive of the presence of two binding components, one with a higher affinity and a lower capacity for the ligand ($K_d = 3.3 \times 10^{-10}$ M, $B_{max} = 0.8$ fmol/mg protein) and the other with lower affinity and higher capacity ($K_d = 10 \times 10^{-7}$ M, $B_{max} = 36$ fmol/mg protein). Polyacrylamide gel electrophoresis of mineralo- and glucocorticoid binding in the cytosol of popliteal artery also showed the presence of the corticosteroid binding components with limited binding capacity. Sucrose density gradient centrifugation of corticosteroid binding in cytosol of femoral artery revealed the presence of dexamethasone binding molecule sedimenting in the 8-9 S region and deoxycorticosterone binding molecules sedimenting in the 7-8 and 8-9 S regions of the gradient. Those results revealed the presence of mineralo- and glucocorticoid receptors in human artery and suggest the possible direct actions of corticosteroids upon human artery. — human artery; gluco- and mineralocorticoid receptors

Direct and indirect effects of corticosteroids upon vascular tissues have been postulated from many experimental and clinical studies. It is known that the initial interaction of steroid hormones with the target tissue is the binding of the hormones to intracellular inactivated receptors, an action which is considered to be common to all the steroids (Grody et al. 1982). The location of steroid receptors in the cells, however, is in dispute (Welshons et al. 1985; Fuxe et al. 1985). A series of works have been aimed at proving the existence of those receptors in arteries of experimental animals (Duval et al. 1977; Kornel et al. 1982a, b) and in cultured aortic cells (Meyer and Nichols 1981; Nichols et al. 1982).

To the best of our knowledge, the presence of steroid receptors in the human vascular system has not been reported except for cultured human arterial smooth

---

Received April 1, 1986; accepted for publication July 8, 1986.

† To whom the correspondence should be addressed.

muscle cells (Meyer et al. 1985). The present investigation was undertaken to assess mineralo- and glucocorticoid binding in cytosol of human arteries from fresh surgical materials and umbilical cord.

MATERIALS AND METHODS

Human arteries

All arteries were obtained from fresh surgical materials and delivered placentas. Popliteal artery was obtained from a 40-year-old male whose left femur had been amputated because of malignant fibrous histiocytoma of the left popliteal fossa. Femoral artery was obtained from a 50-year-old male on whom disarticulation of the right hip joint had been performed because of periosteal osteosarcoma of the right lateral femoral condyle. Inferior mesenteric arteries were obtained from a 45-year-old male for polyacrylamide gel electrophoresis (PAGE) and from a 50-year-old male for dextran coated charcoal (DCC) assay. In both cases, the Miles' abdominoperitoneal resection had been performed due to adenocarcinoma of the rectum. Umbilical arteries were obtained from the placentas of 36 and 32 year old females of full term delivery. None of the patients had any history of hypertension or endocrine disorders.

Steroids

(1, 2-³H)-11-Deoxycorticosterone (DOC) (58.6 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). (1, 2, 4-³H)-Dexamethasone (40 Ci/mmol) was purchased from Amersham International (Arlington Heights, IL, USA). Non-tritiated steroids were purchased from Sigma Chemical Co. (St. Louis, MO, USA). ³H-DOC was used for the mineralocorticoid receptor assay because it has been demonstrated that the relative affinity of DOC for animal arterial receptor is higher than aldosterone (Kornel et al. 1982a). Other chemicals were purchased from Sigma Chemical Co.

Preparation of cytoplasmic extracts

All the arteries were dissected out immediately after amputation and delivery. They were cleaned of most blood, fat and surrounding connective tissues at 4°C, washed with cold physiological saline and then stored at -80°C for further examinations in surgical materials or immediately for use in umbilical arteries. Part of the arteries were fixed in 5% formalin solution for histological examination and no carcinoma infiltrations or hypertensive changes were observed in the arteries used.

Frozen arteries were thawed and homogenized with four to five volumes of cold (4°C) TEMG buffer A; pH 7.4, 10 mM Tris-HCl, 1 mM EDTA, 3 mM sodium azide, 10 mM sodium molybdate, 30% (v/v) glycerol, 0.01% (v/v) monothioglycerol, and 100 µg/ml of protease inhibitors (leupeptin, antipain, chymostatin, elastatinal purchased from Protein Research Foundation, Osaka), for the DCC assay: TEMG buffer B; TEMG buffer A without protease inhibitors and sodium molybdate, for PAGE assay: or TEDS buffer; pH 7.4, 10 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 10 mM sodium molybdate, for sucrose density gradient centrifugation analysis. Umbilical arteries were thawed and homogenized with four to five volumes of cold TTEM buffer; pH 7.4, 20 mM TES, 1.5 mM EDTA, 20 mM sodium molybdate and 0.1% monothioglycerol. The homogenate was centrifuged at 100,000 × g at 4°C for 60 min. The supernatant (cytosol) was then drawn off with a Pasteur pipette and immediately used for further analysis.

Separation of bound and free ³H-steroids

Prepared cytosol was incubated with increasing concentrations of H-steroids for 4 hr at 4°C either alone or in the presence of 10⁻⁵ M unlabelled steroids in frozen arteries, and for 2 hr at 23°C with or without 200 fold of unlabelled steroids in umbilical arteries. The

conditions were determined in order to yield maximum binding and temperature-dependent exchange assay (Sasano and Martin 1985) was applied in umbilical arteries since the ratio of occupied binding sites in the receptor is expected to be high due to increased concentrations of steroid hormones in the circulation. The DCC assay was employed to the separation of bound and free fractions in surgical materials and largely based on Rosseau et al. (1972). Sephadex G-25 ($V_t=2.5$ ml) was used for the separation in umbilical arteries and radioactivity and protein concentration were determined on aliquots of the protein bound fraction.

The dissociation constant (K_d) and number of maximum binding sites (B_{max}) were determined by Scatchard analysis. Curvilinear Scatchard plots in DOC binding were resolved into two lines using the "BMDP" (Program 3R-non-linear regression). Protein was measured by the method of Lowry et al. (1951) using BSA as a standard.

PAGE assay of cytoplasmic steroid receptor

The procedure was based largely on that of Miller et al. (1975). Briefly, cytosols of arteries prepared in TEMG buffer B were incubated with 2×10^{-8} M ^3H -steroids for 4 hr at $4^\circ C$, either alone or in the presence of 1.0×10^{-6} M homologous non-tritiated steroids. Separation gel was $T=7\%$, $C=15\%$ in glucocorticoid binding and $T=8.5\%$, $C=15\%$ in mineralocorticoid binding. Stacking gel was $T=3\%$, $C=20\%$. ^3H -steroid receptor complexes were activated for 20 min at $23^\circ C$ prior to application. Electrophoresis was performed at $4^\circ C$ until the bromphenol blue dye had reached 0.5 cm from the bottom of the gel tubes. The gels were then sliced into 1 mm pieces by a gel slicer, soaked into scintillator composed of 100 volumes of solution A (100 mg POPOP and 4 g PPO per liter of toluene), 10 volumes of Protosol (NEF-935, NEN) and 1 volume of ammonia hydroxide (30% v/v), left overnight at room temperature and counted for their radioactivity.

Sucrose density gradient centrifugation

The assay procedure including determination of marker enzyme activities was based largely upon Martin and Ames (1961) and Toft and Gorski (1966). Briefly, 100 μl of cytosol in TEDS buffer was reacted with 2×10^{-8} M ^3H -steroids for 12 hr at $4^\circ C$, either alone or in the presence of 1.0×10^{-6} M homologous non-tritiated steroid hormones. After incubation, the labelled cytosols were layered over linear 5-20% (w/v) sucrose gradients in 50 mM Tris-HCl with 1 mM dithiothreitol, 10 mM sodium molybdate, 1 mM EDTA, 100 $\mu g/ml$ each of protease inhibitors, and marker enzymes (porcine malate dehydrogenase, yeast alcohol dehydrogenase, beef liver catalase purchased from Boehringer-Mannheim).

The gradients were centrifuged for 40 hr at $\times 80,000$ g at $4^\circ C$. After centrifugation, the gradients were harvested from the top and collected in 1 ml fractions. 500 μl of each fractions was pipetted into scintillation vials with 10 ml of Scintisol EX-2 and radioactivity was measured in an Aloka liquid scintillation counter.

RESULTS

Scatchard analysis of ^3H -dexamethasone binding

Scatchard plots of ^3H -dexamethasone binding in femoral artery (Fig. 1) and umbilical arteries showed a linear pattern suggestive of a single Dexamethasone binding component. Binding parameters obtained from the least square method revealed $K_d=4.3 \times 10^{-9}$ M and $B_{max}=42$ from/mg protein in femoral artery and $K_d=8.2 \times 10^{-9}$ M and $B_{max}=90$ fmol/mg protein in umbilical artery.

Scatchard analysis of ^3H -deoxycorticosterone binding

The Scatchard plot of ^3H -DOC binding in inferior mesenteric artery (Fig. 2)

Fig. 1. Scatchard analysis of ^3H -dexamethasone binding in cytosol of femoral artery (50-year-old male) 200 μl of incubated cytosol was mixed with 500 μl of DCC solution (0.01% Dextran T-70, 10 mM Tris-HCl, 1% Norit), the mixture was shaken intermittently for 5 min and then centrifuged at $1,000\times g$ for 30 min at 4C. 500 μl of aliquots of supernatant were pipetted into scintillation vials together with an aqueous scintillator (Dottite Scitisol EX-2) for counting of their radioactivity.

Fig. 2. Scatchard analysis of ^3H -DOC binding to cytosol of inferior mesenteric artery (50-year-old male). A and B are seen in the text.

shows a curvilinear pattern suggestive of the presence of two DOC binding components. The plot was resolved into two straight lines using "BMDP" program; Line A, representing a higher affinity and a lower capacity for DOC

($K_d = 3.3 \times 10^{-10}$ M, $B_{max} = 0.8$ fmol/mg protein) and Line B, representing a lower affinity and a higher capacity for DOC ($K_d = 10 \times 10^{-7}$ M, $B_{max} = 36$ fmol/mg protein).

PAGE assay

The results are shown in Fig. 3A. and Fig. 3B. In both experiments, PAGE showed steroid binding components of limited binding capacity. Relative mobility of the fastest component in the glucocorticoid binding study was consistent with the peak of radioactivity found in the glucocorticoid binding study of swine liver (unpublished data).

Sucrose density gradient centrifugation

The sedimentation patterns of the dexamethasone and DOC binding components of human femoral artery cytosol are shown in Fig. 4A and B respectively. Fig. 4A clearly demonstrates the presence of ^3H -dexamethasone-binding macromolecule sedimenting in the 8-9S region of the gradients. Fig. 4B shows the presence of ^3H -DOC binding molecules. The DOC-binding molecule with higher radioactivity sedimented in the 7-8 S region of the gradient. The DOC-binding molecule with lower radioactivity sedimenting in the 8-9 S region of the gradient. The sedimentation rate of the latter component is almost the same as that of the

Fig. 3. ^3H -dexamethasone (A) and ^3H -DOC (B) binding analysis in cytosol of popliteal artery, 40-year-old male (A), and inferior mesenteric artery, 45-year-old male (B), by means of polyacrylamide gel electrophoresis. The first two slow components in Fig. 3A may be aggregates of ^3H -dexamethasonereceptor complexes. Radioactive peak of the fastest component in Fig. 3A corresponds in relative mobility to that of activated ^3H -dexamethasone binding component of swine liver cytosol. The peaks of radioactivity are suppressed by the presence of $\times 50$ of homologous, nontritiated steroid hormones.

Fig. 4. Sucrose density gradient centrifugation of steroids binding macromolecules.

Dexamethasone (A) and DOC (B) binding molecules were separated by sucrose density gradient centrifugation of cytosol obtained from femoral artery. The arrows and curve above the profile indicate the position of various marker enzymes; alcohol dehydrogenase (150,000 m.w.), catalase (250,000 m.w.) and malate dehydrogenase (70,000 m.w.).

^3H -dexamethasone binding macromolecule.

DISCUSSION

The mineralo- and glucocorticoid bindings in human artery fit the criteria of steroid receptor, exhibiting a high affinity and a low capacity for each of the corticosteroid hormones. Steroid hormone receptors have been demonstrated in arterial tissues of animals (Duval et al. 1977; Harder and Coulson 1979; Osborne-Pellegrin et al. 1980; Kornel et al. 1982 a, b; Lin et al. 1982) and have been reported to consist of two or three components. Kornel and Kanamarlapudi (1985) reported that mineralo- and glucocorticoid receptors were present only in smooth muscle layer not in intima of bovine aorta. Meyer and Nichols (1981) and Nichols et al. (1982) identified two classes of mineralocorticoid binding sites in cultured smooth-muscle cells and in fibroblasts of rat aorta: corticoid receptor I has a higher affinity and a lower capacity for aldosterone, deoxycorticosterone and corticosterone and lower affinity for steroids of glucocorticoid action; and corticoid receptor II has a lower affinity and a higher capacity for aldosterone, deoxycorticosterone and corticosterone and higher affinity for steroids of glucocorticoid action. This binding activity is similar to that of Type I (true mineralocorticoid receptor) and II (glucocorticoid receptor) binding in the kidney (Funder et al. 1973).

The Scatchard plot of DOC binding in inferior mesenteric artery was curvilinear (Fig. 2), suggesting the presence of at least two different classes of binding sites; one with a higher affinity and a lower capacity and the other with a lower affinity and a higher capacity for DOC. Sucrose density gradient centrifugation of DOC binding to cytosol of femoral artery (Fig. 4B) also suggests the presence of two different classes of binding components: one with the 7–8 S sedimentation rate and another with the 8–9S sedimentation rate. The sedimentation rate of the larger binding component was almost equal to that of the glucocorticoid binding molecule in the same cytosol. This glucocorticoid binding molecule had a sedimentation rate equal to that of the glucocorticoid receptor molecule of the hepatoma tissue culture cells (Failla et al. 1975). The smaller DOC binding component of Fig. 4B had a sedimentation rate almost equal to that of the mineralocorticoid receptor in rat kidney cytosol (Grekin and Sider 1980). Those steroid binding components in sucrose density gradient centrifugation are considered to represent gluco- and mineralocorticoid receptors in human artery but it requires further investigations to clarify this point.

Kornel et al. reported the better preservation of unstable mineralocorticoid binders of bovine aorta cytosol in the presence of molybdate (Kornel et al. 1982b). For preventing the loss of steroid binding activity as much as possible, we have employed proteases inhibitors which were reported to inhibit the breakdown of steroid hormonereceptor complexes (Wilson and French 1979; Hazato and Murayama 1981) in addition to sodium molybdate, especially to stabilize mineralocorticoid binding in DCC assay and sucrose density gradient.

It is known that steroid hormones initially must bind to intracellular receptors in target tissue in order to exert their biological effects, although the location of receptors in cell is in dispute as described above. The presence of gluco- and mineralocorticoid receptors in cytosol of human artery may suggest that these hormones act directly on arterial wall and that they regulate various biological phenomena through constriction or dilatation of vascular wall. There is a growing body of evidence which supports this hypothesis (Swingle et al. 1959; Kalsner 1969; Beilin et al. 1970; Jones and Hart 1975; Friedman and Nakashima 1978; Onoyama et al. 1979; Coghlan et al. 1980; Jones 1981). The analysis of vascular corticosteroid receptors provides a new aspect in understanding the pathophysiology of various disorders, especially pathogenesis of hypertensive disorders.

Acknowledgments

We appreciated Dr. Tetsuo Honma (Dept. of Orthopedic Surgery, Tohoku University School of Medicine, Sendai) for having provided the opportunities to obtain femoral and popliteal arteries, Dr. Akio Ouchi (the First Department of Surgery, Tohoku University School of Medicine, Sendai) for having provided the access to inferior mesenteric arteries, and Dr. Merry Shermann (Memorial-Sloan-Kettering Cancer Center, New York, NY, USA), Dr. Maria I. New and Drs. Kumiko O. Martin (Department of Pediatrics, Cornell Medical

Center-The New York Hospital, New York, NY, USA) for their critical and helpful comments.

References

- 1) Beilin, L.J., Wade, D.N., Honour, A.J. & Cole, T.J. (1970) Vascular hyper-reactivity with sodium loading and with desoxycorticosterone induced hypertension in the rat. *Clin. Sci.*, **39**, 793-810.
- 2) Coghlan, J.P., Butkus, A., Denton, D.A., Graham, W.F., Humphery, T.J., Scoggins, B. A. & Whitworth, J.A. (1980) Steroid receptors and hypertension. *Circulat. Res.*, **46**, Suppl. I, 88-93.
- 3) Duval, D., Funder, J.W., Devynck, M.A. & Meyer, H. (1977) Arterial glucocorticoid receptors: The binding of tritiated dexamethasone in rabbit aorta. *Cardiovasc. Res.*, **11**, 529-535.
- 4) Failla, D., Tomkins, G.M. & Santi, D.V. (1975) Partial purification of a glucocorticoid receptor. *Proc. nat. Acad. Sci. USA*, **72**, 3849-3852.
- 5) Friedman, S.M. & Nakashima, M. (1978) Evidence for enhanced Na transport in hypertension induced by DOCA in the rat. *Canad. J. Physiol. Pharmacol.*, **56**, 1029-1035.
- 6) Funder, J.W., Feldman, D. & Edelman, I.S. (1973) The roles of plasma binding and receptor specificity in the mineralocorticoid action of aldosterone. *Endocrinology*, **92**, 994-1004.
- 7) Fuxe, K., Wikström, A-C., Okret, S., Agnati, L.F., Härfstrand, A., Yu, Z-Y., Granholm, L., Zoli, M., Vale, W. & Gustafsson, J-Å. (1985) Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel- and diencephalon using a monoclonal antibody against rat liver glyocorticoid receptor. *Endocrinology*, **117**, 1803-1812.
- 8) Grekin, R.J. & Sider, R.S. (1980) Aldosterone receptor assay in rat kidney cytosol. *J. Steroid Biochem.*, **13**, 835-837.
- 9) Grody, W.W., Schrader, W.T. & O' Malley, B.W. (1982) Activation, transformation, and subunit structure of steroid hormone receptors. *Endocr. Rev.*, **3**, 141-163.
- 10) Harder, D.R. & Coulson, P.B. (1979) Estrogen receptors and effects of estrogen on membrane electrical properties of coronary vascular smooth muscle. *J. Cell Physiol.*, **100**, 375-382.
- 11) Hazato, T. & Murayama, A. (1981) Protection of steroid hormone receptors by protease inhibitors. *Biochem. biophys. Res. Commun.*, **98**, 488-493.
- 12) Jones, A.W. (1981) Kinetics of active sodium transport in aortas from control and deoxycorticosterone hypertensive rats. *Hypertension*, **3**, 631-640.
- 13) Jones, A.W. & Hart, R.G. (1975) Altered ion transport in aortic smooth muscle during deoxycorticosterone acetate hypertension in the rat. *Circulat. Res.*, **37**, 333-341.
- 14) Kalsner, S. (1969) Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circulat. Res.*, **24**, 383-395.
- 15) Kornel, L. & Kanamarlapudi, N. (1985) Mineralocorticoid (MC) and glucocorticoid (GC) receptors (R) in bovine aorta: MCR's are present only in the smooth muscle. Program and Abstracts, 67th Annual Meeting, The Endocrine Society, p. 249.
- 16) Kornel, L., Ramsey, C., Kanamarlapudi, N., Travers, T. & Packer, W. (1982a) Evidence for the presence in arterial walls of intracellular-molecular mechanism for action of mineralocorticoids. *Clin. exp. Hyper. -Theory and Practice*, **A4(9 & 10)**, 1561-1582.
- 17) Kornel, L., Kanamarlapudi, N., Travers, T., Taff, D.J., Patel, N., Chen, C., Baum, R. M. & Raynor, W.J. (1982b) Studies on high affinity binding of mineralo- and glucocorticoids in rabbit aorta cytosol. *J. Steroid Biochem.*, **16**, 245-264.
- 18) Lin, A.L., McGill, H.C., Jr. & Shain, S.A. (1982) Hormone receptors of the Baboon

- cardiovascular system. *Circulat. Res.*, **50**, 610-616.
- 19) Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
  - 20) Martin, R.G. & Ames, B.N. (1961) A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. biol. Chem.*, **236**, 1372-1379.
  - 21) Meyer, W.J. & Nichols, N.R. (1981) Mineralocorticoid binding in cultured smooth muscle cells and fibroblasts from rat aorta. *J. Steroid Biochem.*, **14**, 1157-1168.
  - 22) Meyer, J.W., Scott, B.A. & Lawrence, B. (1985) Aldosterone and dexamethasone binding in human arterial smooth muscle cells. Program and Abstracts, 67th Annual Meeting, The Endocrine Society, p. 248.
  - 23) Miller, L.K., Diaz, S.C. & Sherman, M.R. (1975) Steroid-receptor quantitation and characterization by electrophoresis in highly cross-linked polyacrylamide gels. *Biochemistry*, **14**, 4433-4443.
  - 24) Nichols, N.R., Hall, C.E. & Meyer, W.J., III (1982) Aldosterone binding sites in aortic cell cultures from spontaneously hypertensive rats. *Hypertension*, **4**, 646-651.
  - 25) Onoyama, K., Bravo, E.L. & Tarazi, R.C. (1979) Sodium, extracellular fluid volume, and cardiac output changes in the genesis of mineralocorticoid hypertension in the intact dog. *Hypertension*, **1**, 331-336.
  - 26) Osborne-Pellegrin, M., Coutard, M., Weill, D., Pearce, P. & Funder, J.W. (1980) Intimal lesions in rat caudal artery; ablation, replacement, and receptor studies on the protective effects of estrogen. *Circulat. Res.*, **46**, Suppl. I, 103-105.
  - 27) Rosseau, G.G., Baxter, J.D. & Tomkins, G.M. (1972) Glucocorticoid receptors: relations between steroid binding and biological effects. *J. molec. Biol.*, **67**, 99-115.
  - 28) Sasano, H. & Martin, K.O. (1985) Development of exchange assay of mineralocorticoid receptor. Program and Abstracts 67th Annual Meeting, The Endocrine Society, p. 85.
  - 29) Swingle, W.W., DaVanzo, J.P., Glenister, D., Crossfield, H.C. & Wagle, G. (1959) Role of gluco- and mineralocorticoids in salt and water metabolism of adrenalectomized dogs. *Amer. J. Physiol.*, **196**, 283-286.
  - 30) Toft, D. & Gorski, J. (1966) A receptor molecule for estrogens: isolation from rat uterus and preliminary characterization. *Proc. nat. Acad. Sci. USA*, **55**, 1574-1581.
  - 31) Welshons, W.V., Krummel, B.M. & Gorski, J. (1985) Nuclear localization of unoccupied receptors for glucocorticoids, estrogens, and progesterone in GH₃ cells. *Endocrinology*, **117**, 2140-2147.
  - 32) Wilson, E.M. & French, F.S. (1979) Effects of proteases and protease inhibitors on the 4.5 S and 8 S androgen receptor. *J. biol. Chem.*, **254**, 6310-6319.
-