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High-affinity angiotensin receptors in rat adrenal medulla

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Summary

Angiotensin II receptors have been quantitated in single rat adrenal medullas by incubation of tissue sections with ^{125}I -[Sar¹]-AII, autoradiography with exposure to ^3H -sensitive Ultrofilm, computerized densitometry and comparison with ^{125}I -labelled standards. Rat adrenal medulla contains a single class of high affinity AII receptors with a K_a of $0.84 \pm 0.02 \times 10^9 \text{ M}^{-1}$ and a B_{max} of $3259 \pm 502 \text{ fmol/mg}$ protein, one of the highest densities in AII receptors found in rat tissues. These observations provide evidence for a local site of action of AII in the release of adrenal medullary catecholamines.

angiotensin II receptors; rat adrenal medulla; receptor autoradiography

Introduction

Release of adrenal medullary catecholamines is under neural and humoral control. Among the many factors involved, angiotensin II (AII) has been proposed to play a modulatory role [1]. AII directly releases catecholamines from adrenal medulla [2], probably through an action on specific AII receptors [3]. In addition, AII could affect catecholamine release from the adrenal medulla through central actions mediating splanchnic nerve stimulation, or indirectly through corticosteroid release [1].

The development of new highly sensitive autoradiographic techniques for receptor quantitation [4,5] allowed us to study AII receptor kinetics in discrete rat brain nuclei and peripheral tissues from individual animals [6]. We report the presence of single

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class, high-affinity AII receptors in rat adrenal medulla. A preliminary study on the presence of AII receptors in this tissue was recently presented [7].

Material and Methods

Rats were sacrificed by decapitation and adrenal glands immediately removed and frozen by immersion in isopentane at -30°C . Frozen, $16\ \mu\text{m}$ thick sections were cut in a cryostat at -14°C and thaw-mounted onto subbed glass slides, prepared by dipping them in a 0.5% gelatin solution containing 0.05% chromium potassium sulfate. Binding sites for AII were labelled *in vitro* by incubation with ^{125}I -[Sar¹]-AII (a gift from Dr. M. Khosla, Cleveland Clinic, Cleveland, OH, iodinated by a modified chloramine-T method at Meloy Laboratories Inc., Springfield, VA, specific activity 1666 Ci/mmol). Tissue sections from four individual animals were preincubated in duplicate for 15 min at 20°C in 5 ml of 10 mM sodium phosphate buffer, pH 7.4, containing NaCl (120 mM), Na₂EDTA (5 mM), bacitracin (0.1 mM) and bovine serum albumin (0.2%) [8] and then incubated for 60 min in fresh buffer with concentrations of ^{125}I -[Sar¹]-AII from 25 pM to 11 nM. Non-specific binding was determined in the presence of unlabelled AII (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from 0.125 to 50 μM . After incubation the slides were washed four times (60 s each) with ice-cold 50 mM Tris-HCl buffer, pH 7.56, and dried under a cold stream of air.

Sets of ^{125}I -standards were prepared as described for ^3H -labelled standards [4,6]. Known amounts of increasing concentration of ^{125}I -AII (specific activity 1280 Ci/mmol, New England Nuclear, Boston, MA) were thoroughly mixed with brain tissue aliquots previously ground to a paste, placed as blocks of tissue on microtome specimen holders, and frozen on dry ice. Tissue sections, $16\ \mu\text{m}$ thick, were cut in a cryostat at -14°C and thaw-mounted onto subbed glass slides. Parallel sets of standards were used for determination of protein concentrations [9] and radioactivity.

Incubated tissue sections and ^{125}I -standards were placed in cassettes (CGR Med. Corp., Baltimore, MD) and opposed against [^3H]Ultrofilm (LKB Industries, Rockville, MD) at room temperature for one day for concentrations from 0.6 to 11 nM and 2 days for concentrations from 25 pM to 0.3 nM. The films were developed at 20°C for 4 min with undiluted D19 Kodak developer and optical densities were quantitated by computerized densitometry [4]. There was a linear relationship between the ln of the optical densities read from the standards and the ln of the concentration of radioactivity after different exposure times of [^3H]Ultrofilm to the standards (Fig. 1, right panel). The optical densities observed in adrenal gland sections were related to the concentration of radioactivity present by comparison with standard curves generated by processing sets of standards with each of the autoradiograms (Fig. 2, right panel) [6], and were determined by interpolating the optical densities read in tissue areas on the straight line obtained from the ln-ln standard curve.

A characteristic curve of the film is shown in Fig. 1, left panel. This curve is generated by the semi-log plot of the optical densities read from the standards vs. the exposure time of the film and the amount of radioactivity actually present in the

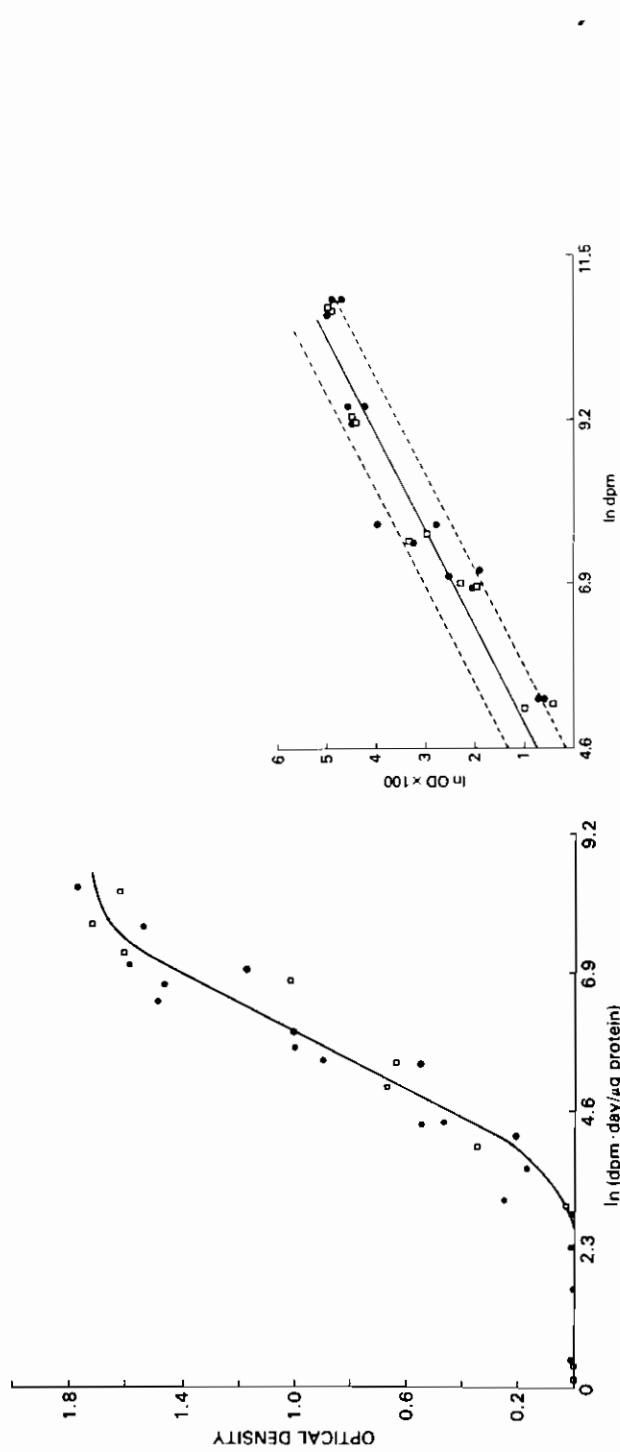


Fig. 1. Quantitative autoradiographic technique for ^{125}I -ligands. Right panel: A \ln - \ln plot of O.D. vs. radioactivity (dpm/ μg protein) in standards. Exposure times were 1 (\square) and 2 (\bullet) days. Each point represents the average of three optical density readings from autoradiographs generated from triplicate standard curves and prepared for each film utilized for the quantitation of AII receptors. Dashed lines represent the standard error of estimate of X from Y . The general equation of the straight line was, for 24 pairs of data: $Y = 0.757 X - 2.76$; $r = -0.84$; $F = 68.4$ ($P < 0.001$). Left panel: Characteristic curve of ^{125}I -trofilm with ^{125}I -standards. Optical densities are plotted as a function of \ln of dpm/ μg protein \times exposure time. Each point represents the average of three optical density readings from autoradiograms generated from triplicate standard curves. Exposure times were 1 (\square) and 2 (\bullet) days.

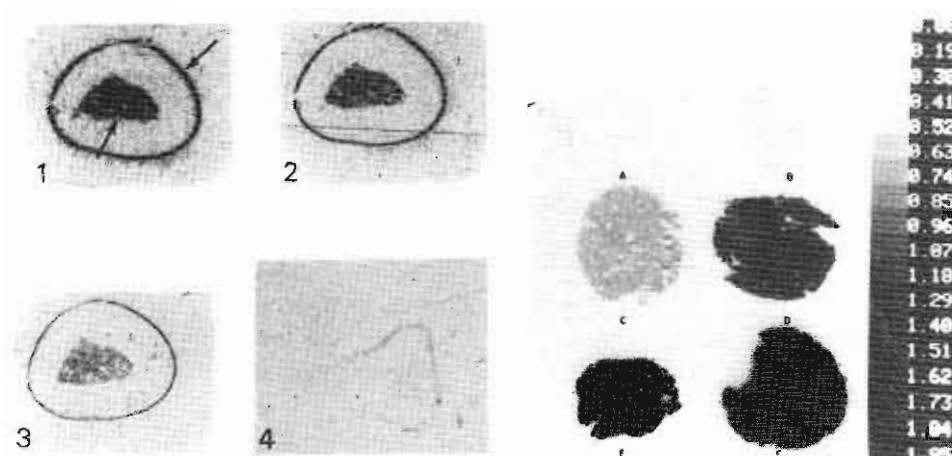


Fig. 2. Angiotensin II receptors in rat adrenal gland. Left panel: 1, 2 and 3. Rat adrenal gland ($16 \mu\text{m}$ sections) incubated with 5.0, 1.25 and 0.32 nM ^{125}I -[Sar¹]-AII. Arrows point to zona glomerulosa and to adrenal medulla. 4. Adjacent section incubated with 5 nM ^{125}I -[Sar¹]-AII and 25 μM unlabelled AII. Right panel: Autoradiographic image of ^{125}I -standards with computerized densitometry. Each section contains a different amount of radioactivity per mg of protein. In dpm: A, 9.17; B, 20.8; C, 72.8; D, 200.5; E, 536.4; F, 1008.0.

standards. This relationship was similar to that established for ^3H -labelled standards [4]. The slope of the curve gives the measure of the photographic contrast. Film exposure times were adjusted according to the receptor concentration in different areas, so that the optical densities read were no lower than 0.3 units and no higher than 1.6 units, since a linear relationship exists between optical densities at this range and $\text{dpm}/\mu\text{g}$ of protein \times time of exposure. Such a relationship makes it easy to discriminate between small differences in concentration of radioactivity, i.e., between different concentrations of binding sites for a particular structure [6]. Optimum film exposure times for each adrenal area and for each ligand concentration were determined in preliminary experiments [6,13].

Binding data obtained from sections of single rat adrenal glands were analyzed and Scatchard plots were produced with the LIGAND computer program [10]. All data are presented as the mean \pm S.E.M.

Experimental results

Incubation of adrenal gland sections with ^{125}I -[Sar¹]-AII showed that AII receptors were highly localized in the adrenal medulla and in the zona glomerulosa of the adrenal cortex (Fig. 2, left panel). The addition of unlabelled AII resulted in almost a total displacement of the ^{125}I -[Sar¹]-AII binding in both structures (Fig. 2, left panel, 4, and Fig. 3).

Saturation curves and Scatchard analysis were performed in duplicate sections, with ^{125}I -[Sar¹]-AII concentrations ranging from 25 pM to 11 nM (Fig. 3). In the adrenal medulla, AII receptors had a K_a of $0.84 \pm 0.02 \times 10^9 \text{ M}^{-1}$, and a B_{max} of

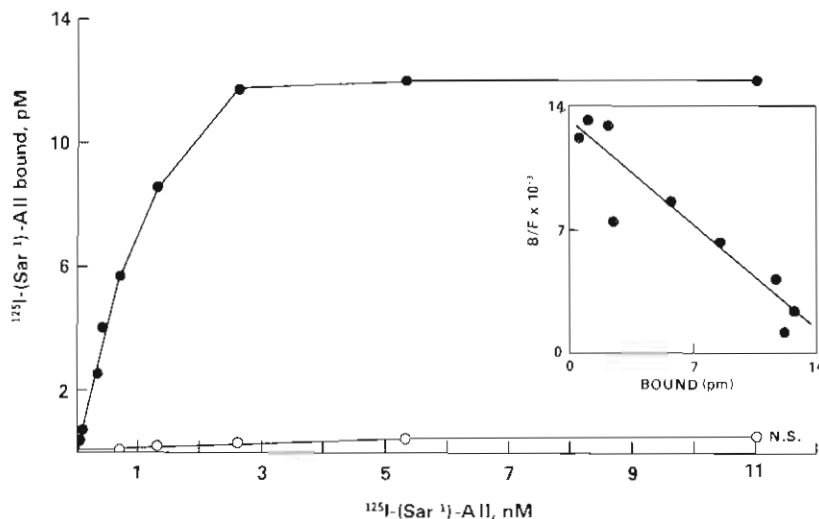


Fig. 3. Saturation curve and Scatchard analysis of specific ^{125}I -[Sar¹]-AII binding to rat adrenal medullary sections. Tissue sections were incubated for 60 min with ^{125}I -[Sar¹]-AII (concentrations from 25 pM to 11 nM). Non specific (N.S.) binding was determined in the presence of unlabelled AII (from 0.125 to 50 μM). Data represent a typical experiment which was replicated four times. Inset: Scatchard analysis of the same data. The binding capacity was 3259 ± 502 fmol/mg protein and the K_a was $0.843 \pm 0.02 \times 10^9 \text{ M}^{-1}$, as calculated by computer analysis of binding data using the program LIGAND [10].

3259 ± 502 fmol/mg protein (Fig. 3). In the adrenal cortex, AII receptors were localized in the zona glomerulosa, with a K_a of $0.80 \pm 0.06 \times 10^9 \text{ M}^{-1}$ and a B_{max} of 5650 ± 579 fmol/mg protein.

Discussion

Precise quantitation is essential for studies on the kinetic properties and physiological regulation of receptors. Autoradiographic techniques coupled with computerized densitometry allow the quantitative determination of receptor characteristics with the added advantage of precise anatomical localization. The method requires determination of the so called characteristic curve of the film (Fig. 1, left panel) obtained by comparison of the optical densities read from the film vs. the product of the exposure time and the amount of radioactivity present in the standards. The slope of the curve gives a measure of the photographic contrast. For tissues with high AII binding, such as the rat adrenal medulla, the time of exposure of the [³H]Ultrafilm should be relatively short (1 and 2 days in the present experiments) to prevent saturation of the film. Optimum time of exposure can be determined in preliminary experiments for each particular structure to be studied. Optimum contrast is obtained with optical densities between 0.6 and 1.4 units. Molar quantities of ligand bound per mg of tissues are determined by interpolating the optical densities read by densitometry in the straight line obtained from the \ln of optical densities– \ln of dpm per mg protein standard curve (Fig. 1, right panel) [4,6].

With this method, we were able to perform saturation curves and Scatchard analysis of AII receptors in single rat adrenal glands. Both the affinity and total number of AII receptors in rat adrenal zona glomerulosa are of the same order of magnitude as the ones obtained with rat adrenal cortex membrane preparations [11]. In addition, our studies revealed the existence of a single class of saturable, high affinity binding sites for AII in the rat adrenal medulla. The concentration of AII receptors in this tissue is one of the highest for AII receptors reported so far and is higher than that recently reported for adrenal medullary AII binding sites determined by classical membrane binding techniques [12]. This discrepancy can be explained by the author's use of a crude adrenal medullary membrane preparation, by the contamination of the freshly dissected rat adrenal medullary tissue by adrenal cortex, containing no AII receptors, and by possible loss of binding sites during the homogenization process. Similar differences in AII receptor numbers were observed between autoradiographic techniques and membrane binding methods in the rat pituitary gland [14].

Our observations indicate a possible physiological role for AII locally at the adrenal medullary level, and complement previous pharmacological studies demonstrating a direct effect of AII on catecholamine release from adrenal medulla [2,3]. Our data suggest that the effects of peripheral AII on adrenal medulla may have an important local component, in addition to the postulated centrally mediated splanchnic stimulation [13]. That the adrenal medullary AII receptors may play an important physiological role is demonstrated by their increased B_{max} in adult spontaneously hypertensive rats, a change coincident with the animals' increased capacity for catecholamine release (Plunkett and Saavedra, submitted).

Studies are in progress to clarify further the physiological regulation of adrenal medullary AII receptors and their precise cellular localization.

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