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^{3}H-Spiroperidol (Spiperone) Binding Sites in Rat Adrenal Glomerulosa Cells

Michihiro MATSUKI, Seikoh NISHIDA, Masaharu HORINO, Noriko ADACHI, Masaya YONEDA and Hideki OYAMA

Division of Endocrinology, Department of Medicine, Kawasaki Medical School, Kurashiki 701-01, Japan

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ABSTRACT. ^{3}H-spiperone, a dopaminergic antagonist, was used to study binding sites in rat adrenal glomerulosa membrane. The equilibrium dissociation constant (Kd) and binding capacity for ^{3}H-spiperone binding were 2.2 nM and 268 fmol/mg protein, respectively. Determination of the Kd by kinetic studies provided a value of 2.6 nM, which corresponded closely to the Kd estimated by equilibrium studies. In a study of the subcellular distribution of dopamine receptors in adrenal glomerulosa cells, ^{3}H-spiperone binding activity at the interface of density 1.14 to 1.16 accounted for 60% of the total activity in all fractions. These dopaminergic binding sites in adrenal glomerulosa cells may modulate aldosterone secretion induced by antidopaminergic agents.

Key words : ^{3}H-spiroperidol — Dopamine receptor — Rat adrenal glomerulosa cells

Dopamine may have an important role in the regulation of aldosterone secretion in adrenal gland. Dopamine itself has been shown to inhibit angiotensin-mediated aldosterone secretion.[1] Metoclopramide, a dopamine antagonist, stimulates aldosterone biosynthesis both in rat[2,3] and man.[4–7] These dopaminergic effects on aldosterone secretion may be mediated by adrenal dopamine receptors. The peripheral dopamine receptor consists of two distinct subclasses, which are called D_1 and D_2 according to pharmacological characteristics.[8] The presence of dopamine receptors in the rat,[9] calf[10] and bovine[11] glomerulosa cells has been demonstrated. On the contrary, there are reports showing only indirect influences of dopamine on aldosterone production in adrenal glomerulosa cells,[12-14] and Bevilacqua *et al.*[10] suggested that dopaminergic regulation of aldosterone was not mediated by D_2 receptor. In this report, the identification of dopamine receptors in rat adrenal glomerulosa cells and its characteristics are described.

MATERIALS AND METHODS

Preparation of crude membrane fraction from rat adrenal glomerulosa cells

Adult male Wistar rats weighing 150–300 g were decapitated and adrenal glands were immediately removed. Adrenal glands were separated into capsular (glomerulosa) and decapsular portions, as reported by Giroud *et al.*[15] The capsular portion was cut with a razor blade into fine pieces. These pieces

松木道裕，西田聖幸，堀野正治，安達倫子，米田正也，尾山秀樹

were homogenized in 2 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a Teflon pestle homogenizer. The homogenate was centrifuged at 750×g for 10 minutes, and the supernatant was recentrifuged at 39,000×g for 15 minutes. The final pellet was homogenized in 10 volumes of cold 50 mM Tris-HCl buffer.

^{3}H-Spiperone bind

^{3}H-Spiperone (17 Ci/mmol; Amersham) was diluted with 50 mM Tris-HCl buffer. For determination of the relative binding of ^{3}H-spiperone to adrenal glomerulosa membrane, 50 μl of 50 mM Tris-HCl buffer was mixed with 50 μl of crude membrane fraction containing 20-40 μg protein and 0.25-8 nM ^{3}H-spiperone. Each sample was incubated in triplicate for 15 minutes at 22°C. At the end of the incubation, samples were filtered through Whatman GF/B glass fiber filters (Whatman, Inc., Clifton, NJ, USA), which were washed twice with 4 ml of cold 50 mM Tris-HCl buffer under vacuum. The radioactivity of the filters were counted by liquid scintillation spectrometry. Specific binding was defined as the amount of ^{3}H-spiperone bound to membranes in the absence of dopamine (total binding) minus the amount of ^{3}H-spiperone bound in the presence of 10^{-2} M dopamine (non-specific binding), as described by Dunn *et al.*[9] Scatchard analysis of the data was performed to determine the dissociation constant (Kd) and site numbers.[16] The protein concentration of the final homogenate was determined using Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA, USA), with bovine serum albumin (Seikagaku Kogyo, Co., LTD., Tokyo) for standard.

Subcellular fractions

Subcellular localization of dopamine receptors in adrenal glomerulosa cells was investigated by means of discontinuous sucrose density gradient centrifugation, carried out according to a method similar to that described by Poirier *et al.*[17] The 39,000×g final pellet was suspended in buffer and adjusted to a density of 1.18 with sucrose. This suspension was layered in a five layer discontinuous sucrose gradient of densities 1.14, 1.16, 1.18, 1.20 and 1.22. The gradient was centrifuged at 120,000×g for 4 hr in a Hitachi 65 P (RPS 65T roter) centrifuge. Each layer was collected, and assayed for receptor binding.

RESULTS

Specific binding was saturable at 0.25-8 nM ^{3}H-spiperone and represented 50-60% of the total binding. A Scatchard plot of the specific binding of ^{3}H-spiperone to adrenal glomerulosa membranes revealed a straight line with Kd of 2.2 nM and a relative binding of 268 fmol/mg protein (Fig. 1). The kinetic binding of ^{3}H-spiperone to adrenal glomerulosa membrane is shown in Fig. 2. Specific binding achieved equilibrium within 10 minutes, and reversibly dissociated by addition of excess of dopamine. The association constant was estimated to be $K_1 = 0.0275$ nM^{-1} min^{-1}. The rate of dissociation of ^{3}H-spiperone under the standard conditions (adding 10^{-2} M dopamine to prevent the rebinding of dissociated ^{3}H-spiperone) was calculated to be $K_2 = 0.0727$ min^{-1}. The Kd derived from the rate constants ($Kd = K_2/K_1$) was 2.6 nM.

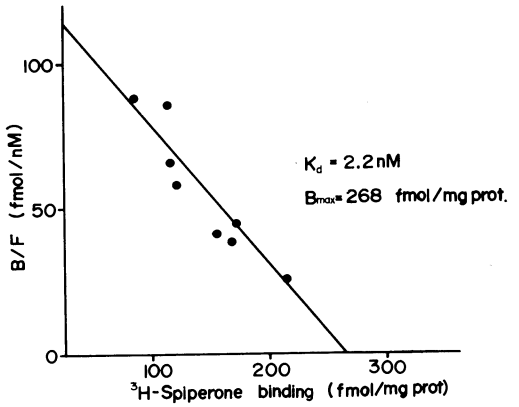

Fig. 1. A Scatchard analysis of specific ^{3}H-spiperone binding to rat adrenal glomerulosa membranes. Each value is an average of triplicate determinations.

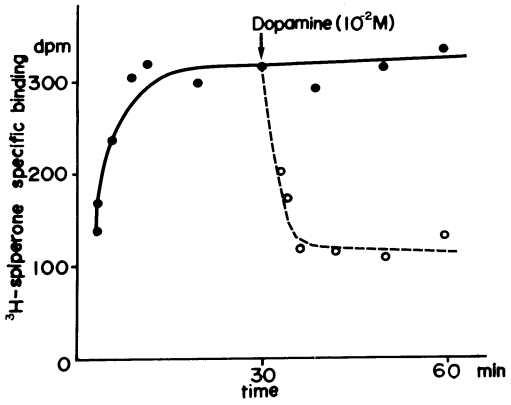

Fig. 2. Time course of ^{3}H-spiperone binding in rat adrenal glomerulosa cells. Each point is an average of triplicate determinations. The ^{3}H-spiperone concentration is 4 nM. (●) ; total binding of ^{3}H-spiperone. (○) ; 10^{-2} M dopamine added to prevent the rebinding of dissociated ^{3}H-spiperone.

TABLE 1. Distribution of ^{3}H-spiperone specific binding activity in adrenal glomerulosa subcellular fractions.

Fractions (sucrose densities)	^{3}H-spiperone specific binding (fmol/mg protein)
Homogenate	62.6
1.14-1.16	378.6
1.16-1.18	48.6
1.18-1.20	122.9
1.20-1.22	97.5
Pellet	0

The result of the study of subcellular distribution of ^{3}H-spiperone binding is shown in Table 1. The binding activity present at the interface of densities 1.14 to 1.16 constituted approximately 60% of the total activity.

DISCUSSION

In the present study, the specific binding of spiperone to dopaminergic sites was calculated as the difference between the amount of ^{3}H-spiperone alone (total binding) and that in an excess of dopamine (non-specific binding). In the measurement of non-specific binding, d-butaclamol, haloperidol, chlorpromazine, etc. are usually used rather than dopamine. These drugs inhibit ^{3}H-spiperone binding at nanomolar concentrations in brain dopamine receptors.[18] Dunn *et al.* have shown that there is no difference in the displacement of ^{3}H-spiperone binding between that by dopamine and that by d-butaclamol. Furthermore, our kinetic binding study showed the rapid displacement of 4 nM ^{3}H-spiperone binding to rat adrenal glomerular cells by 10^{-2} M dopamine. These data agree with the specific binding of spiperone to dopamine receptors determinations using dopamine.

We identified dopamine receptors in rat adrenal glomerulosa cells by using ^{3}H-spiperone. The dissociation constant for ^{3}H-spiperone was 2.2 nM and 2.6 nM by Scatchard plot analysis and kinetic binding study, respectively. Its binding was rapid and rapidly reversible. The nanomolar order of the dissociation constant indicates that dopamine receptor in the glomerulosa cells should be classified as D_2 (not cyclase linked)[8]. However, it is now of considerable problem whether these peripheral D_2 dopamine receptors are identical to the central D_2 receptors or not, as defined by Kebabian *et al.*[8] Missale *et al.*[11] suggested that D_2 receptors in bovine striatum and its adrenal cortex, are identical as using ^{3}H-sulpiride radioligand.

Appreciable ligand activity was present at the interfaces of densities 1.18 to 1.20 and 1.20 to 1.22. The ligand activities could be attributed to some contaminating plasma membranes, as suggested by Poirier *et al.*[17] ^{3}H-Spiperone binding activity was approximately 6-fold higher in the plasma membrane fraction than in the original homogenate. The demonstration of maximal binding activity in plasma membrane fraction from rat adrenal gland agrees with similar localization in rat pituitary cells.[19] In these maximal binding activity fraction of dopamine, adenylate cyclase activity is markedly enriched. Recently, Missale *et al.*[20] demonstrated a presence of D_1 (adenylate cyclase linked) receptor in rat adrenal cortex, coexisting of D_2 receptor.

The relationship between the effect of dopaminergic antagonists and that of agonists on aldosterone biosynthesis in adrenal glomerulosa cells is complex. *In vivo* findings have shown that metoclopramide increases aldosterone production, but *in vitro* studies have shown either no effect or an inhibitory effect on aldosterone biosynthesis.[12,13] Braley *et al.*[14] demonstrated in an *in vitro* study that angiotensin II-mediated aldosterone production increased by adding dopamine and metoclopramide, in contrast to inhibitory effect of metoclopramide on aldosterone production.

In the present study, rat adrenal glomerulosa cells contain ^{3}H-spiperone binding sites using by equilibrium and kinetic studies and its pharmacological characteristics provide D_2 receptor subclass.

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