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Copurification of β-Adrenergic Catecholamine Receptors and Hormone-Sensitive Adenylate Cyclase with Uterine Smooth Muscle Plasma Membranes (40937)[1]

J. FREDERICK KRALL AND STANLEY G. KORENMAN

Molecular Endocrinology Laboratory and Department of Medicine, UCLA–San Fernando Valley Program, Veterans Administration Medical Center, Sepulveda, California 91343

Abstract. A putative plasma membrane fraction isolated from rat uterine smooth muscle was substantially enriched in both β-adrenergic catecholamine receptors as detected by radioligand binding methods and adenylate cyclase. Receptors for adrenergic catecholamines as well as series E prostaglandins were functionally coupled to the enzyme in the purified membranes by a GTP-requiring process. Localization of receptors and enzymes to the same subcellular compartment suggest that in myometrium, as in other target tissues, β-adrenergic catecholamines and perhaps series E prostaglandins exert their effects by regulating cAMP production.

In the uterine smooth muscle cells of rats and rabbits both the positively and negatively inotropic functions of adrenergic catecholamines are mediated by specific receptors which have been characterized by radioligand binding methods (1–3). The action of a different class of potent contractile hormones, the series E prostaglandins (PGE), is likewise receptor mediated (4, 5). The biochemical mechanism of action of the α-adrenergic catecholamines and PGE is not well understood, but in most target tissues receptors for β-adrenergic catecholamines are a regulatory subunit of adenylate cyclase and hence modulate intracellular cAMP levels (6). Despite the apparent ubiquity of the mechanism of action of β-adrenergic catecholamines, the role of cAMP in their relaxing effects on the smooth muscle cell is contested (7, 8).

Using a combination of differential and density equilibrium centrifugation in sucrose, we previously characterized a membrane fraction from the rat myometrium enriched in plasma membrane marker enzyme activity (9). We now demonstrate that both hormone-sensitive adenylate cyclase and β-adrenergic catecholamine receptors are localized to this fraction, further suggesting that this specific class of cell surface receptors has an important function in the regulation of cAMP production in myometrium.

Materials and methods. Uteri from 200- to 250-g Sprague–Dawley rats were trimmed of fat, cut open lengthwise, and scraped free of endometrium in ice-cold normal saline. Well-rinsed muscle strips were then diced with scissors and homogenized with a Brinkman Polytron (PT-10ST generator) for 30 sec at 2 to 4° in 10 vol of 0.25 *M* sucrose, 0.01 *M* Tris–HCl (pH 7.4), 0.01 *M* $MgCl_2$, 0.0025 *M* dithiothreitol and filtered through glass wool. The differential centrifugation fraction richest in plasma membrane-associated enzyme activity was centrifuged to equilibrium on discontinuous sucrose gradients and the fraction which banded at the 20/35% interface collected and centrifugally concentrated as described previously (9).

The plasma membrane-rich pellets were rinsed and resuspended in: (a) 0.05 *M* Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.6), 0.001 *M* EGTA, 10% dimethylsulfoxide (for adenylate cyclase determination) or (b) 0.075 *M* Tris–HCl (pH 7.4), 0.025 *M* $MgCl_2$, 0.0025 *M* dithiothreitol (for β-adrenergic catecholamine receptor assay). The protein concentrations of the resultant suspensions was determined by the method of Lowry *et al.* (10) using bovine serum albumin as a standard after precipitation with 10% per-

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chloric acid, washing (by centrifugation), and finally extraction at 90° with the same dilute acid.

Adenylate cyclase activity was determined as the enzymatic conversion of [α-^{32}P]ATP to [^{32}P]cAMP by purification of the product by successive chromatography on columns of Dowex and aluminum oxide according to our previously published method (11). β-Adrenergic catecholamine receptor concentration of the membranes or other myometrial particulate fractions was determined by specific membrane binding in the presence of 5n*M* (−)-[^{3}H]dihydroalprenolol (^{3}H-DHA) according to Krall *et al.* (3). Under these conditions, 90% of the binding of this saturating concentration of the radioligand is to a single class of high-affinity binding sites and displaced by 1 μM unlabeled (−)-propranolol in particulate fractions.

For the determination of specific ^{3}H-DHA binding in intact myometrium, whole muscle strips were incubated at 23° with 5 n*M* radioactive antagonist in the presence or absence of 1 μM unlabeled (−)-propranolol in 1 ml of 0.05 *M* Tris−HCl (pH 7.4), 0.01 *M* $MgCl_2$, and 0.0025 *M* dithiothreitol and 10 m*M* catechol. After 20 min the muscle strips were removed to 50 ml of 0.01 *M* Tris−HCl (pH 7.4) at 4° and washed by agitating for 5 min. The wash procedure was repeated at the same temperature and the tissue pieces were blotted dry and solubilized overnight in 90% NCS (Amersham) prior to addition of scintillation fluid. Under these conditions, greater than 25% of binding to the intact muscle strips was specific and displaceable by 1 μM unlabeled propranolol.

Results and discussion. Most of the adenylate cyclase activity and all of the β-adrenergic catecholamine receptors present in rat myometrial homogenates is present in a particulate fraction which sediments between 10,000 and 50,000*g* (Table I). When this particulate fraction is centrifuged to equilibrium on discontinuous sucrose gradients as described previously, membranes which band at the 20/35% sucrose interface have an equilibrium density of 1.15, are free of contamination by mitochondrial or nuclear membranes, and enriched in 5′-nucleotidase and Na^+,K^+-ATPase (7). The presence of these marker enzymes and the uniform resistance of other proteins in this fraction to trypsin-proteolysis and salt extraction suggests that the membranes are a homogeneous smooth muscle cell plasma membrane (sarcolemma) preparation. The putative sarcolemmal fraction is also rich in adenylate cyclase activity (Table I), an enzyme which, though described associated with

TABLE I. DISTRIBUTION OF HORMONE RECEPTORS AND ADENYLATE CYCLASE IN RAT MYOMETRIAL SUBCELLULAR FRACTIONS[a]

	Homogenate	10,000*g* pellet	50,000*g* pellet	50,000*g* supernatant	Purified sarcolemma
	β-Adrenergic[b] catecholamine receptor				
Adenylate cyclase activity[c]	N.D.[d]	0.0	30 ± 6	0.0	650 ± 80
Basal	28 ± 2	5 ± 1	43 ± 5	<2	106 ± 24
+ 100 μM ISO	27 ± 4	4 ± 1	39 ± 2	<2	97 ± 31
+ 100 μM PGE_2	28 ± 1	5 ± 2	39 ± 4	<2	103 ± 4
+ 100 μM GTP	45 ± 4	7 ± 1	114 ± 6	<2	156 ± 27
+ GTP + 100 μM ISO	68 ± 1	8 ± 0	167 ± 2	<2	260 ± 42
+ GTP + 100 μM PGE_2	62 ± 6	7 ± 0	133 ± 10	<2	246 ± 12
10 m*M* NaF	114 ± 3	15 ± 2	263 ± 29	<2	1368 ± 60

[a] Results are the mean of four to seven separate determinations ± SEM.
[b] In fmole of ^{3}H-DHA specifically bound per mg of protein.
[c] In pmole of cAMP formed/mg·min.
[d] Not determined.

other cytomembranes, is generally acknowledged as a plasma membrane enzyme in most cells (12–16).

In the myometrium and other β-adrenergic catecholamine target tissues, adenylate cyclase is a complex enzyme composed of the catalytic subunit, the specific hormone receptor regulatory subunit, and a purine nucleotide-dependent subunit or mechanism which couples together the other two components (11, 17). The coupling mechanism is reflected by the enzyme activity dependent on GTP and in myometrium stimulation of adenylate cyclase by the specific β-adrenergic catecholamine agonist isoproterenol as well as PGE has an absolute requirement for GTP or other purine nucleotides (Table I). Increases in adenylate cyclase specific activity as a result of purification of the sarcolemmal fraction were large (14-fold) in the presence of NaF but less than half that in the presence of GTP or either isoproterenol or PGE with GTP. This suggests that components of the hormone sensitive pathway are localized to the sarcolemma, and also that the different components have different labilities in the face of the purification procedure. The catalytic subunit, directly stimulated by NaF, appears the most stable. The following suggests that the β-adrenergic catecholamine receptor is not greatly damaged by isolation, so that the coupling mechanism between the regulatory and catalytic subunits may be the component most sensitive to homogenization and plasma membrane purification in smooth muscle as well as other target tissues (18).

Binding of the radioactive β-adrenergic catecholamine antagonist ^{3}H-DHA to the receptor is impossible to detect in crude homogenates due to the low concentration of specific binding sites compared to total protein concentration. All of the receptors detectable by these radioligand binding methods are associated with the myometrial particulate fraction which sediments between 10,000 and 50,000g, however (Table I and Ref. (3)). The pellet contains nearly all of the specific ^{3}H-DHA binding sites present in myometrium when total organ concentration calculated from particulate binding is compared to binding to intact muscle strips (Table II), suggesting that few receptors are lost as a result of homogenization. When the β-adrenergic catecholamine receptor concentration of the sarcolemmal fraction is compared to that of the 50,000g pellet, a 20-fold increase in receptor density is achieved by the purification procedure, comparable to that achieved based on NaF-stimulated adenylate cyclase activity. Isolation of the receptor-rich membranes occurs at the expense of yield, however, since aggregation of sarcolemmal elements with other cytomembranes, probably mitochondrial (9), accounts for appreciable plasma membrane loss reflected by recovery of less than 20% of the myometrial receptors with the purified membranes (Table II). Losses of a similar magnitude are associated with the purification of adenylate cyclase with the sarcolemmal fraction (not shown).

The high-affinity binding sites for radioactive antagonists in myometrial particulate fractions of rat and rabbit fulfill the strict criteria anticipated for the receptors which mediate β-adrenergic catecholamine function in the uterine smooth muscle cell (2, 3). Their copurification with a sarcolemmal fraction rich in hormone-dependent adenylate cyclase activity suggests but does not prove that, consistent with evidence in other target tissues, β-adrenergic catecholamines exert their physiological effects through the regulation of cAMP production in myometrium. Moreover, since myometrial adenylate cy-

TABLE II. β-ADRENERGIC CATECHOLAMINE RECEPTOR CONCENTRATIONS OF RAT MYOMETRIUM

Preparation	Receptor concentration (fmole of ^{3}H-DHA specifically bound per myometrium)
Intact muscle strip[a]	170 ± 61
50,000g[b] particles	118 ± 20
Sarcolemma[b]	20 ± 7

[a] Values are the means ± SD of 12 separate muscle strips.

[b] Calculated from the data in Table I.

clase is sensitive not only to β-adrenergic catecholamines but also to PGE, receptors for this class of agonist, previously localized by ^{3}H-PGE binding methods to a membrane fraction in hamster uterus (4), may also be sarcolemmal. As a consequence, some functions of β-adrenergic catecholamines and series E prostaglandins in the regulation of uterine motility may occur through their interaction in the regulation of adenylate cyclase.

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