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REGULATION OF ADRENOCORTICAL CARDIOTONIC STEROID PRODUCTION BY DOPAMINE AND PKA SIGNALING

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1. ABSTRACT

There is growing evidence that the adrenal cortex is the source of cardiotonic steroid (CS) regulators of sodium, potassium-ATPase (NKA). The control of adrenocortical production CS may play a critical role in mediating renal and vascular responses involved in arterial hypertension. Dopamine (DA) controls renal NKA by direct regulatory phosphorylation and indirectly by modification of aldosterone release. In the present studies, Y-1 adrenocortical cell cultures which have been shown to produce a cardiotonic steroid indistinguishable from the known vertebrate steroid, marinobufagenin (MBG), were treated with various agents to stimulate or antagonize dopamine signaling pathways. We demonstrate that Y-1 cells express both pharmacological types of dopamine receptor (DA1 and DA2). Treatment of Y-1 cells with DA stimulated MBG production in a dose range similar to that shown to inhibit aldosterone production by the adrenal cortex. Experiments with specific DA1 and DA2 receptor agonists and antagonists were performed and allowed us to attribute the DA stimulatory effect to a DA1 type receptor. The DA stimulatory effect on MBG depended on protein kinase A (PKA) and could be blocked by Rp-cAMPS. Although both basal and forskolin-stimulated progesterone production by Y-1 cells were profoundly inhibited in Y-1 cell lines expressing the dominant negative type I regulatory subunit of PKA, both basal and forskolin-stimulated MBG production were demonstrated in these lines. This evidence suggests a possible role of DA1 signaling through cAMP-mediated activation of the type II PKA holoenzyme in the adrenal cortex.

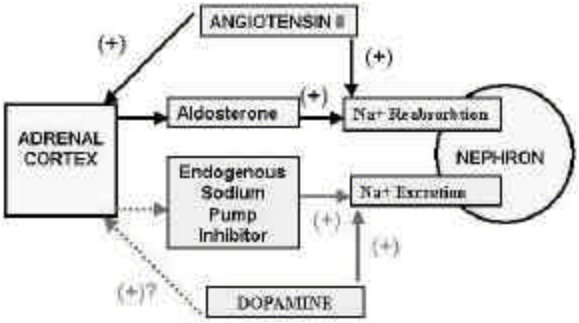

Figure 1. Hypothesis: Adrenal action of dopamine on cardiotonic steroid secretion reinforces the nephronal role of dopamine and angiotensin II to regulate sodium excretion/reabsorption.

2. INTRODUCTION

The primary driving force of renal sodium reabsorption is sodium, potassium-ATPase (NKA), the sodium pump. Evidence from Mendelian forms of hypertension emphasize the critical role of excess renal sodium reabsorption in the generation of elevated blood pressure (1). Numerous systems converge to regulate renal sodium excretion. Because most natriuretic systems either are without direct effect on blood pressure, or are anti-hypertensive, there has been interest in endogenous cardiotonic steroid inhibitors of NKA because the generalized actions of such inhibitors can include both reduction of renal sodium reabsorption and increased vascular tone and elevated blood pressure (2-5). Cardiotonic steroids have also been demonstrated to stimulate proliferation of renal epithelial cells (6) and myocytes (7-11) at concentrations too low to cause shifts in transmembrane ion gradients. Recent evidence indicates that coupling of NKA to Src by cardiotonic steroids transactivates the epidermal growth factor receptor and activates MAP kinase signaling pathways implicated in these proliferative responses (6, 12). Thus, such compounds may play an important role to link excess renal sodium retention to blood pressure elevation in which inhibition of renal NKA facilitates electrolyte balance, vasoactive effects contribute to the induction of pressure-natriuresis, and signaling by MAP kinase pathways supports adaptation of the mechanical properties of the cardiovascular system to the attendant increased mechanical stress.

Growing evidence reveals that the adrenal cortex is the site of biosynthesis of one or more cardiotonic steroids that may function to restore sodium balance and elevate blood pressure by inhibiting NKA (13). We have shown that endogenously produced cardiotonic steroids are the product of an adrenocortical steroidogenesis pathway that is independent of cholesterol side-chain cleavage (14, 15). We have also shown that the chemical identity of one of these steroids is indistinguishable from the known vertebrate cardiotonic steroid marinobufagenin (MBG) (15-18).

Dopamine plays a key role in the regulation of sodium excretion. This role occurs at multiple levels and is integrated with control by other regulatory agents. The adrenal cortex takes up L-dopa from plasma and converts it into DA (19). Dopamine receptor gene expression and receptor protein are present in mammalian adrenal cortex (20). Ligand binding studies further support the presence of dopaminergic receptor sub-types in the adrenal cortex (20-23). Adrenocortical DA receptors localize to the zona glomerulosa, the site of production of mineralocorticoids. The DA- and cAMP-regulated phosphoprotein, DARPP, a critical mediator of dopaminergic signaling in other tissues, is also present in the adrenal cortex (24). The DA receptor pharmacological subtype 2 has been implicated directly in the control of aldosterone (ALDO) production and DA interaction with this receptor also antagonizes AII-stimulated ALDO production (21-23). Dopamine also inhibits ALDO production during volume expansion (25). Intra-renal actions of dopamine include lowered sodium reabsorption in proximal tubule by inhibition of NKA activity (26). Dopamine regulation of renal sodium excretion is integrated with control by other systems. Renal dopamine opposes both the short- and long-term anti-natriuretic effects of angiotensin (27, 28). Angiotensin II stimulates the activity of proximal tubule NKA and this effect is completely abolished in the presence of DA or its second messenger cAMP (29). Together, this evidence places dopamine at the apex of a cascade of regulatory events, some occurring in the kidney and others through alterations in adrenocortical function, that converge on renal mechanisms of sodium balance.

The purpose of the present work was to investigate the role of DA in regulation of MBG production in adrenocortical cells. We hypothesized that DA increases MBG production by adrenal cortex by a protein kinase A-mediated signaling pathway. Such an adrenal action to increase NKA inhibitor production may reinforce other direct and indirect actions of DA to produce net renal sodium loss. Our hypothesis is presented schematically in figure 1.

3.MATERIALS AND METHODS

3.1. Cell culture

Mouse adrenocortical tumor cells (Y-1) were purchased from American Type Culture Collection. Cells were grown to near confluence in 12-well plates in DMEM/F12 (1:1) supplemented with 15% horse serum and 2% fetal bovine serum at 37°C in the presence of 5% CO_2. In each experiment, cells were pre-incubated for 16 hr in serum-free medium followed by 2hr stimulation with agents including DA, DA1 and DA2 receptor agonists and antagonists and ACTH. Conditioned medium was collected and stored at -20°C prior to extraction and analysis. Some studies employed Y-1 cell lines stably transfected to express a dominant negative regulatory subunit (type I) of protein kinase A (Y-1/RIAB). Empty selection vector transfected cells were used as controls (Y-1/neo). The steroidogenic phenotypes of the transfected cell lines have been fully characterized and previously reported (30).

Table 1. PCR Primer sequences and products sizes

Gene name	Specific primers	Nested primers	Product size bp[1]
D1	F 5′-CTA CAG GAT TGC CCA GAA GC-3′ R 5′-TAC CCC CAT GAT CAC AGA CA-3′	F 5′-GCA TCT CAG CTT TGG AGA GG-3′ R 5′-CCT CTT AAA GGA CAT CTT AAA GGA A-3′	190/145
D2	F 5′-CTG TAC CTT CGG GGG AAA AT-3′ R 5′-ACC TCC AAC TTC AGC TCC AA-3′	N/A	200
D3	F 5′-CTT TGG CAA CGG TCT GGT AT-3′ R 5′-TGA CAT CCA GGG TGA CAA AA-3′	N/A	203
D4	F 5′- GTG TGT TGG ACG CCT TTC TT-3′ R 5′-GTT CTT TCA GCA GCG GSG AC-3′	F 5′-CTG TGT CCG GCT TGC TTC-3′ R 5′-GCG GAA GAC ACT TCG AAA CT-3′	198/160
D5	F 5′-TCC AAC TCA ATT GGC ACA GA-3′ R 5′-GCG CGT GTA GGT CAC TAT CA-3′	F 5′-AGA GGG CCT GCT GTC CAA T-3′ R 5′-AGA AGC TGA TGA GCG ACG AG-3′	203/164

F: forward primer, R-: everse primer [1] Specific/nested

3.2. RNA extraction and reverse-transcriptase-polymerase chain reaction (RT-PCR)

Extraction of total RNA from Y-1 cells was done with RNAqueous-4PCR RNA isolation Kit (Ambion, Austin, TX). RNA concentration was estimated from absorption at 260 nm. Samples were stored at -80°C. Primer and PCR product sizes are shown in the table 1: *PCR primer sequences and product sizes*. For the RT step, 3μg of total RNA was dissolved in 20 μl of reaction mixture (GeneAmp RNA PCR Kit, Applied Biosystems, Foster City CA.) and kept at 37°C for 15 minutes. For the PCR step, the resulting RT mixture was transferred into reaction buffer that contained specific primers. PCR involved 35 cycles of 95°C, 60°C, 72°C for 1 minute each in a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA). PCR products were visualized by ethidium bromide staining after electrophoresis on 3% agarose gels and size of products estimated from adjacent size standards. The specificity of PCR products obtained was confirmed by performing a second round of PCR using the initial products with nested primers.

3.3. Immunoassays

MBG cross-reactive material in conditioned culture medium was measured in C18 extracted samples using ELISA as described (15). Samples were tested in the ELISA for their ability to inhibit the binding of rabbit MBG antibody to solid phase-bound MBG (immobilized conjugate of MBG-3-glycoside to RNAse; 0.02 μg of conjugate in 0.1ml of bicarbonate-buffered saline per well). The sensitivity of immunoassay was 0.05 ng/L. The cross-immunoreactivity of marinobufagenin antibody was (%): proscillaridin A, and progesterone and pregnenolone all <0.1; marinobufagenin 100; digitoxin 3.0; bufalin 1.0; digoxin and cinobufagin 1.0; ouabain 0.1; prednisone, spironolactone, Progesterone content of conditioned medium was measured by a specific radioimmunoassay which has been previously described (15).

3.4. Statistical Analysis

The results are expressed as mean±SEM. Statistical significance was determined by t test assuming equal variances. A value of $p<0.05$ was considered to be significant.

3.5. Materials

MBG antibody was the kind gift of Dr. A. Bagrov, National Institute on Aging, Baltimore, MD. Bromocriptine, SKF-38393 and spiperone were generously provided by Dr. M. Lokhandwala, the University of Houston, TX. Dopamine, SCH-23390, AII and all other reagents were obtained from Sigma Chemical Co. Fetal Bovine serum, DMEM, F12 and horse serum were obtained from GIBCO-BRL.

4. RESULTS

4.1. RT-PCR analysis of dopamine (D1-D5) receptor gene expression in Y-1 adrenocortical cells

To address the question of whether adrenocortical MBG production may be regulated by DA and modified by angiotensin II it was necessary to determine whether expression of DA and angiotensin receptors is preserved in Y-1 adrenocortical cells. Figure 2 illustrates results of RT-PCR analysis of DA (D1 through D5) receptor expression in Y-1 cells. RT-PCR analysis showed that Y-1 adrenocortical cells express genes encoding both dopamine pharmacological receptor subtype DA1 (D1 and D5 genes) and subtype DA2 (D4 gene). The identity of RT-PCR products was further verified by nested PCR (figure 2).

4.2. Role of dopaminergic system in regulation of MBG production

Effect of DA on MBG production in adrenocortical cells is shown in figure 3. Basal level of MBG production was 32.8±3.1 ng/L and increased up to 48.7±5.6 ng/L ($p<0.04$) in the presence of 0.1μM of DA and up to 49.2±3.2 ng/L ($p<0.005$) in the presence of 1μM of DA. To determine whether a specific DA receptor subtype mediates the stimulatory effect of DA on MBG production, experiments were performed with specific DA1 and DA2 receptor agonists and antagonists. The results are shown in figure 4. In this set of experiments basal MBG production was 14.5±0.8 ng/L (basal levels of MBG production commonly vary over time and at different levels of culture density in these fast growing adrenocortical tumor cells) and increased in the presence of 100 nM of DA to 24.6±1.4 ng/L ($p<0.001$). The DA stimulatory effect was abolished by DA1 receptor antagonists, SCH-23390 (100 nM), but not by DA2 receptor antagonist, spiperone (100 nM). DA1 receptor agonist SKF-38393 (100 nM), but not DA2 agonist bromocriptine (100 nM) stimulated MBG production (22.7±3.3 ng/L; $p<0.002$). This indicates that DA effects on MBG production are mediated by DA1 receptors.

4.3. Second messenger pathway

The classical signaling pathway for D1-like receptors leads to activation of adenylate cyclase, increased

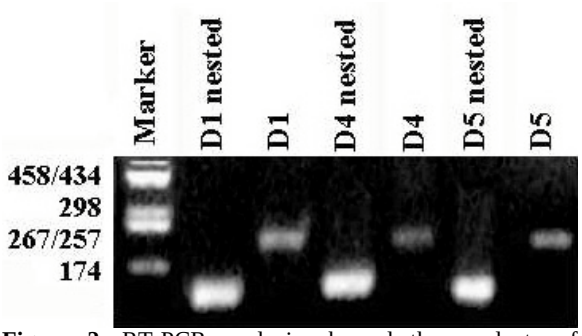

Figure 2. RT-PCR analysis showed the products of expected size for D1A, D4A and D5A specific primers (A). The identity of PCR products obtained with specific primers was confirmed by PCR with nested primers (B). No products were detected for D2 and D3 (data not shown).

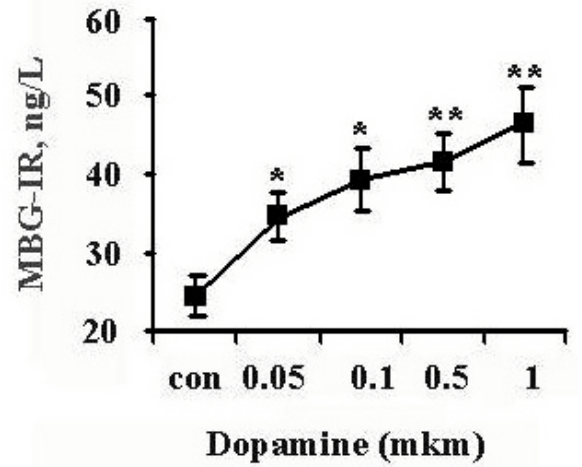

Figure 3. Effect of dopamine on MBG production in Y-1 adrenocortical cultures. Cells were incubated for 2 hr in basal conditions and in medium containing 0.1 μM and 1μM of dopamine. MBG level was measured by ELISA in C-18 extracted conditioned medium. Data are presented as mean ± SEM; n=6 culture dishes; *p<0.05, **p<0.01 vs control.

levels of cAMP, and PKA activation (31). PKA may either directly phosphorylate a target protein or initiate a cascade of phosphorylation events and activation of the DA and cAMP-regulated phosphoprotein, DARPP32, an inhibitor of protein phosphatase 1 (32). The objective of these experiments was to investigate if the cAMP/PKA signaling pathway is involved in MBG regulation.

We used the specific inhibitor of the activation of PKA, Rp-cAMPS to determine whether PKA signaling is involved in regulation of MBG production by dopamine. DA-stimulated MBG production was completely abolished by Rp-cAMPS ($p<0.01$) (figure 5). In order to determine whether PKA signaling involves transduction pathways involving the PKA-I holoenzyme or the PKA-II pathway we studied two stably transfected Y-1 clones expressing either the neomycin resistance gene (Y-1/neo) or a cAMP-dependent protein kinase type I regulatory subunit harboring mutations in both sites A and B of the cAMP-binding domain (Y-1/RIAB). Expression of this mutated RI has been previously shown to markedly impair PKA-dependent control of Y-1 steroidogenesis resulting from cholesterol side-chain cleavage (30). We measured progesterone production in these two lines to demonstrate impairment of cholesterol side-chain cleavage-mediated steroidogenesis and its control by PKA in the Y-1/RIAB line. As expected, the adenyl cyclase activator, forskolin, stimulated progesterone production in the control (Y-1/neo) cell line. Both basal and forskolin-stimulated progesterone release were essentially undetectable in Y-1/RIAB (figure 6A, left panel). Forskolin stimulated MBG production in the Y1/neo cell line, further supporting involvement of PKA signaling in the control of MBG production. In contrast with progesterone production, basal MBG production was readily detected in Y1/RIAB cells and was further stimulated by forskolin. This indicates that MBG production is not controlled by PKA-I signaling, but may be controlled by a PKA-II pathway (figure 6A, right panel). ACTH is a master controller of adrenocortical steroidogenesis and is also able to activate PKA signaling (33). At present, there is no knowledge of whether such activation drives PKA-I, PKA-II or both pathways. Signaling by ACTH of increased side-chain cleavage leading to progesterone accumulation was observed in Y-1/neo, but was absent in Y-1/RIAB (figure 6B, left panel) confirming the role of PKA-I in ACTH-mediated activation of side-chain cleavage. ACTH stimulated MBG production on both Y-1/neo and Y-1/RIAB cell lines in a manner analogous to forskolin stimulation. This result may be explained if, like forskolin, ACTH can activate pathways leading to stimulation of both PKA-I And PKA-II.

5. DISCUSSION

This work was done to probe the hypothesis that a set of mechanisms known to regulate mineralocorticoid secretion in adrenal cortex has opposing effects on the production of adrenocortical cardiotonic steroid (MBG) (figure 1). Body sodium balance is precisely regulated by modulatory factors released from intra- and extrarenal sources. The effects of anti-natriuretic and natriuretic factors are coordinated and an intact DA system is important for the maintenance of sodium homeostasis and normal blood pressure (34). We report here evidence that regulation in the adrenal cortex of the endogenous sodium pump inhibitor, MBG, is under dopaminergic control.

Evidence for a role of DA in the adrenal cortex has come from *in vivo* studies of the control of secretion of the sodium-retaining hormone aldosterone in both humans and experimental animals. Administration of the DA2 antagonist, metoclopramide, in both rats and humans was shown to increase plasma aldosterone levels without modifying any of the known stimulators of the hormone release, an effect that was blocked by intravenous infusion of DA (35-37). *In vitro* studies with freshly isolated adrenal glomerulosa cells demonstrated that activation of DA2 receptors resulted in inhibition of angiotensin II-induced aldosterone secretion but did not modify hormone release under basal conditions or after stimulation by adrenocorticotropic hormone (38). These data indicated

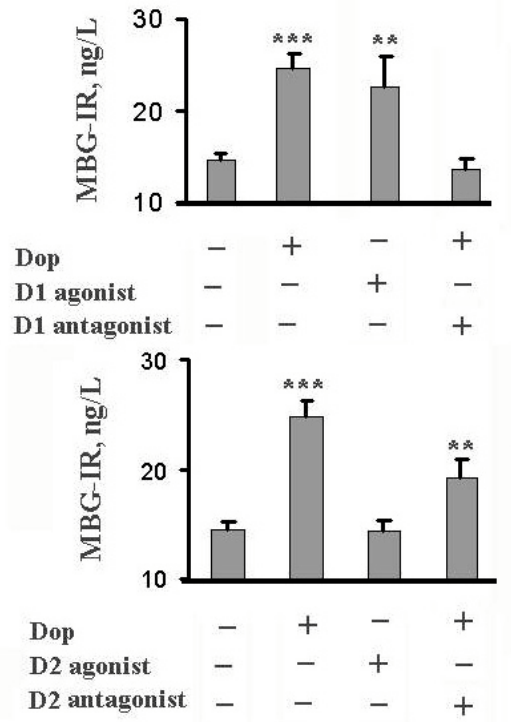

Figure 4. Cells were incubated for 2 hr in basal conditions or stimulated with dopamine alone (0.1μM); dopamine (0.1μM) in presence of D1 or D2 receptors antagonists (5μM); or with dopamine D1 and D2 receptors agonists (0.1μM). MBG level was measured by ELISA in C-18 extracted conditioned medium. Data are presented as mean ± SEM; n≥5 culture dishes; ***p<0.001, **p<0.01 vs control.

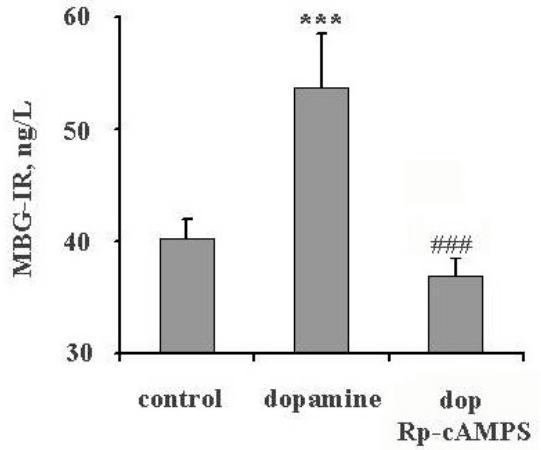

Figure 5. Effect of protein kinase A inhibitor Rp-cAMPS on dopamine-stimulated MBG production. Cells were incubated for 2 hr in basal conditions or stimulated with dopamine alone (0.1μM) or with dopamine (0.1μM) in presence of specific inhibitor of activation of protein kinase A, Rp-cAMPS (10μM). MBG level was measured by ELISA in C-18 extracted conditioned medium. Data are presented as mean ± SEM; n=6 culture dishes; *** p<0.001 vs control, ### p<0.001 vs 0.1μM of dopamine.

that the effects of DA on aldosterone secretion are mediated by DA2 receptors in adrenal glomerulosa cells and pointed to selective, functional interaction between DA and angiotensin II in the regulation of the production of aldosterone. Y-1 cells lack the capacity to synthesize mineralocorticoids, preventing us from monitoring the effect of our dopamine treatments on aldosterone production. Although the pharmacological characterization and localization of both DA1 and DA2 receptors in the adrenal cortex has been shown, the physiological role of DA1 receptors in adrenal glomerulosa cells is previously unknown (38). In the present study we have shown that DA can stimulate synthesis of the NKA inhibitor MBG by Y-1 adrenocortical cells. Based on pharmacological analysis with specific agonists and antagonists we have concluded that this stimulatory effect is mediated via DA1 type receptors. We also show evidence that transcripts of both genes encoding DA1-type pharmacological receptors (D1 and D5) are present in Y-1 cells.

In vitro analysis of the transduction pathways activated by DA receptors in adrenocortical glomerulosa cells revealed that D1 receptors are associated with stimulation of adenylyl cyclase (21). D2 receptors have been shown to inhibit cAMP formation and Ca^{2+} influx (39). The effect of DA on MBG production was blocked by specific PKA inhibitor Rp-cAMPS, which suggests that this stimulation may be the result of activation of protein kinase A through the adenylate cyclase signaling pathway. Surprisingly, MBG production was stimulated by forskolin and ACTH in Y-1 cells transfected with a vector expressing the dominant negative form of the protein kinase A regulatory I (RI) subunit. Over-expression of this mutated RI markedly impairs both basal and stimulated side-chain cleavage dependent-steroidogenesis in Y-1 cells (30) which was confirmed in our experiments (Figure 6). However, we observed that basal MBG production persists and can be further stimulated by forskolin. In our previous work we have developed evidence that adrenocortical cardiotonic steroid production is independent of side-chain cleavage (14, 15, 40). Thus, these observations suggest that elimination of PKA type I signaling affects the capacity to generate steroids by reducing expression of critical components of the side-chain cleavage pathway (41), but leaves cardiotonic steroid production and its regulation unimpaired. The cAMP-stimulation of MBG production, but not progesterone production, in Y-1/RIAB cells expressing mutant RI subunit may be explained if MBG biosynthesis pathway is sustained and stimulated by activation of type II PKA holoenzyme (containing RII subunit).

Protein kinase A is a tetramer comprised of two catalytic (C) subunits and two cAMP-binding regulatory (R) subunits. Two families of R subunit isoforms, RI and RII, have been identified that differ significantly with respect to affinity for activators and inhibitors, regulation by phosphorylation, intracellular localization and may direct catalytic activity to different substrates (42). The type I PKA holoenzyme is predominantly cytoplasmic, whereas >75% of the type II holoenzyme is targeted to certain intracellular sites through association of the RII

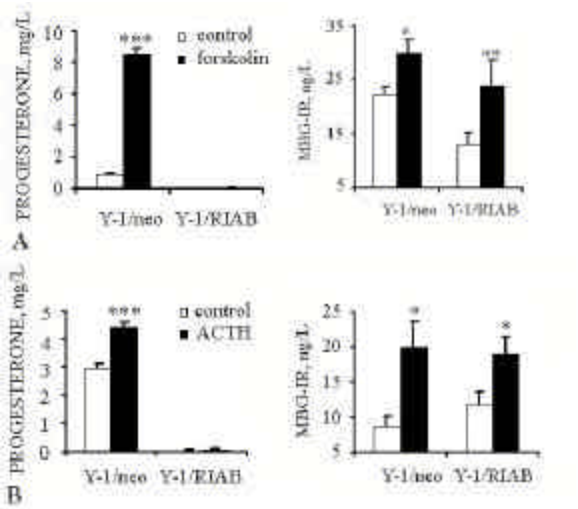

Figure 6. Effect of forskolin (A) and ACTH (B) on MBG production in Y-1 transfected cells. Cells were incubated for 2 hr in basal conditions or stimulated with forskolin (10 mg/ml) or with ACTH (10 nM). MBG level was measured by ELISA in C-18 extracted conditioned medium. Progesterone was measured in conditioned medium by a specific RIA. Data are presented as mean ± SEM; n=6, * = p<0.05, ** = P<0.01, *** = p<0.001, vs control.

subunits with cellular binding proteins known as anchoring proteins (AKAPs) (43). Each anchoring protein contains two classes of binding sites: a conserved "anchoring motif" which binds the regulatory subunit of PKA and a "targeting domain" that directs the sub-cellular localization of the PKA-AKAP complex through association with structural proteins, membranes, or cellular organelles. Sub-cellular targeting, by association with anchoring proteins, has emerged as an important mechanism allowing cells to localize signaling enzymes to sites where they can be accessed optimally by activators and, in turn, interact with particular substrates. Further study will be necessary to clarify the role of PKA subtypes in regulation of adrenocortical sodium pump inhibitor production.

Here we report evidence of how adrenocortical production of the sodium pump inhibitor, MBG, is regulated by dopamine. The dopaminergic signaling pathway is driven by activation of PKA. However, dopamine modulation of MBG production is more likely to involve the type II PKA holoenzyme than the type I holoenzyme. These observations provide insight into a system for the regulation of adrenocortical biosynthesis of the sodium pump inhibitor, MBG, that is fully compatible with the important role of dopamine in the integration of body sodium balance. This work lends further support to the idea that adrenocortical cardiotonic steroid production can play an important role in the integration of fluid and electrolyte balance and cardiovascular function.

6. ACKNOWLEDGMENTS

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7. REFERENCES

1. Karet FE and Lifton RP. Mutations contributing to human blood pressure variation. *Recent Prog Horm Res* 52, 263-276 (1997)
2. Yates NA and McDougall JG. Interaction of exogenous ouabain and chronic mineralocorticoid treatment in the kidney of the conscious sheep. *Clin Exp Pharmacol Physiol* 24, 57-63 (1997)
3. Yates NA and McDougall JG. Effect of volume expansion on the natriuretic response to ouabain infusion. *Ren Physiol Biochem* 18, 311-320 (1995)
4. Juhaszova M and Blaustein MP. Na+ pump low and high ouabain affinity alpha subunit isoforms are differently distributed in cells. *Proc Natl Acad Sci U S A* 94, 1800-1805 (1997)
5. Aperia A. Regulation of sodium/potassium ATPase activity: impact on salt balance and vascular contractility. *Curr Hypertens Rep* 3, 165-171 (2001)
6. Dmitrieva RI and Doris PA. Ouabain is a potent promoter of growth and activator of ERK1/2 in ouabain-resistant rat renal epithelial cells. *J Biol Chem* 278, 28160-28166 (2003)
7. Liu J, Tian J, Haas M, Shapiro JI, Askari A, and Xie Z. Ouabain interaction with cardiac Na+/K+-ATPase initiates signal cascades independent of changes in intracellular Na+ and Ca2+ concentrations. *J Biol Chem* 275, 27838-27844 (2000)
8. Xie Z, Kometiani P, Liu J, Li J, Shapiro JI, and Askari A. Intracellular reactive oxygen species mediate the linkage of Na+/K+-ATPase to hypertrophy and its marker genes in cardiac myocytes. *J Biol Chem* 274, 19323-19328 (1999)
9. Kometiani P, Li J, Gnudi L, Kahn BB, Askari A, and Xie Z. Multiple signal transduction pathways link Na+/K+-ATPase to growth-related genes in cardiac myocytes. The roles of Ras and mitogen-activated protein kinases. *J Biol Chem* 273, 15249-15256 (1998)
10. Scheiner-Bobis G and Schoner W. A fresh facet for ouabain action. *Nat Med* 7, 1288-1289 (2001)
11. Aydemir-Koksoy A, Abramowitz J, and Allen JC. Ouabain-induced signaling and vascular smooth muscle cell proliferation. *J Biol Chem* 276, 46605-46611 (2001)
12. Haas M, Wang H, Tian J, and Xie Z. Src-mediated inter-receptor cross-talk between the Na+/K+-ATPase and the epidermal growth factor receptor relays the signal from ouabain to mitogen-activated protein kinases. *J Biol Chem* 277, 18694-18702 (2002)
13. Schoner W. Endogenous cardiotonic steroids. *Cell Mol Biol (Noisy-le-grand)* 47, 273-280 (2001)
14. Doris PA, Kilgore MW, Durham D, Alberts D, and Stocco DM. An endogenous digitalis-factor derived from the adrenal gland: studies of adrenocortical tumor cells. *Endocrinology* 125, 2580-2586(1989)
15. Dmitrieva RI, Bagrov AY, Lalli E, Sassone-Corsi P, Stocco DM, and Doris PA. Mammalian bufadienolide is synthesized from cholesterol in the adrenal cortex by a

pathway that Is independent of cholesterol side-chain cleavage. *Hypertension* 36, 442-448 (2000)
16. Bagrov AY, Fedorova OV, Dmitrieva RI, Howald WN, Hunter AP, Kuznetsova EA, and Shpen VM. Characterization of a urinary bufodienolide Na+,K+-ATPase inhibitor in patients after acute myocardial infarction. *Hypertension* 31, 1097-1103 (1998)
17. Bagrov AY, Fedorova OV, Dmitrieva RI, French AW, and Anderson DE. Plasma marinobufagenin-like and ouabain-like immunoreactivity during saline volume expansion in anesthetized dogs. *Cardiovasc Res* 31, 296-305 (1996)
18. Fedorova OV, Doris PA, and Bagrov AY. Endogenous marinobufagenin-like factor in acute plasma volume expansion. *Clin Exp Hypertens* 20, 581-591 (1998)
19. Buu NT and Lussier C. Origin of dopamine in the rat adrenal cortex. *Am J Physiol* 258, F287-291 (1990)
20. Aherne AM, Vaughan CJ, Carey RM, and O'Connell DP. Localization of dopamine D1A receptor protein and messenger ribonucleic acid in rat adrenal cortex. *Endocrinology* 138, 1282-1288 (1997)
21. Missale C, Liberini P, Memo M, Carruba MO, and Spano P. Characterization of dopamine receptors associated with aldosterone secretion in rat adrenal glomerulosa. *Endocrinology* 119, 2227-2232 (1986)
22. Missale C, Memo M, Liberini P, and Spano P. Dopamine selectively inhibits angiotensin II-induced aldosterone secretion by interacting with D-2 receptors. *J Pharmacol Exp Ther* 246, 1137-1143 (1988)
23. Stern N, Tuck M, Ozaki L, and Krall JF. Dopaminergic binding and inhibitory effect in the bovine adrenal zona glomerulosa. *Hypertension* 8, 203-210 (1986)
24. Meister B, Schultzberg M, Hemmings HC, Jr., Greengard P, Goldstein M, and Hokfelt T. Dopamine- and adenosine-3',5'-monophosphate (cAMP)-regulated phosphoprotein of 32 kDa (DARPP-32) in the adrenal gland: immunohistochemical localization. *J Auton Nerv Syst* 36, 75-84 (1991)
25. Aguilera G and Catt KJ. Dopaminergic modulation of aldosterone secretion in the rat. *Endocrinology* 114, 176-181 (1984)
26. Aperia A, Bertorello A, and Seri I. Dopamine causes inhibition of Na+-K+-ATPase activity in rat proximal convoluted tubule segments. *Am J Physiol* 252, F39-45 (1987)
27. Chen CJ, Apparsundaram S, and Lokhandwala MF. Intrarenally produced angiotensin II opposes the natriuretic action of the dopamine-1 receptor agonist fenoldopam in rats. *J Pharmacol Exp Ther* 256, 486-491 (1991)
28. Cheng HF, Becker BN, and Harris RC. Dopamine decreases expression of type-1 angiotensin II receptors in renal proximal tubule. *J Clin Invest* 97, 2745-2752 (1996)
29. Aperia A, Holtback U, Syren ML, Svensson LB, Fryckstedt J, and Greengard P. Activation/deactivation of renal Na+,K(+)-ATPase: a final common pathway for regulation of natriuresis. *Faseb J* 8, 436-439 (1994)
30. Lalli E, Melner MH, Stocco DM, and Sassone-Corsi P. DAX-1 blocks steroid production at multiple levels. *Endocrinology* 139, 4237-4243 (1998)
31. Aperia A, Eklof AC, Holtback U, Nowicki S, Sundelof M, and Greengard P. The renal dopamine system. *Adv Pharmacol* 42, 870-873, (1998)
32. Fryckstedt J, Meister B, and Aperia A. Control of electrolyte transport in the kidney through a dopamine- and cAMP-regulated phosphoprotein, DARPP-32. *J Auton Pharmacol* 12, 183-189 (1992)
33. Parker KL and Schimmer BP. Genetics of the development and function of the adrenal cortex. *Rev Endocr Metab Disord* 2, 245-252 (2001)
34. Jose PA, Eisner GM, and Felder RA. Renal dopamine and sodium homeostasis. *Curr Hypertens Rep* 2,174-183 (2000)
35. Carey RM, Thorner MO, and Ortt EM. Effects of metoclopramide and bromocriptine on the renin-angiotensin-aldosterone system in man. Dopaminergic control of aldosterone. *J Clin Invest* 63, 727-735 (1979)
36. Noth RH, McCallum RW, Contino C, and Havelick J. Tonic dopaminergic suppression of plasma aldosterone. *J Clin Endocrinol Metab* 51, 64-69 (1980)
37. Sowers JR, Brickman AS, Sowers DK, and Berg G. Dopaminergic modulation of aldosterone secretion in man is unaffected by glucocorticoids and angiotensin blockade. *J Clin Endocrinol Metab* 52, 1078-1084 (1981)
38. Missale C, Nash SR, Robinson SW, Jaber M, and Caron MG. Dopamine receptors: from structure to function. *Physiol Rev* 78, 189-225 (1998)
39. Gallo-Payet N, Chouinard L, Balestre MN, and Guillon G. Mechanisms involved in the interaction of dopamine with angiotensin II on aldosterone secretion in isolated and cultured rat adrenal glomerulosa cells. *Mol Cell Endocrinol* 81, 11-23, (1991)
40. Doris PA, Hayward-Lester A, Bourne D, and Stocco DM. Ouabain production by cultured adrenal cells. *Endocrinology* 137, 533-539 (1996)
41. Schimmer BP. The 1994 Upjohn Award Lecture. Molecular and genetic approaches to the study of signal transduction in the adrenal cortex. *Can J Physiol Pharmacol* 73, 1097-1107 (1995)
42. Michel JJ and Scott JD. Akap mediated signal transduction. *Annu Rev Pharmacol Toxicol* 42, 235-257 (2002)
43. Colledge M and Scott JD. AKAPs: from structure to function. *Trends Cell Biol* 9, 216-221 (1999)

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