[Frontiers in Bioscience 10, 2257-2262, September 1, 2005]

ENDOGENOUS DIGITALIS-LIKE LIGANDS AND Na/K-ATPase INHIBITION IN EXPERIMENTAL DIABETES MELLITUS

Yakov Y. Bagrov, Natalia B. Manusova, Irina A. Egorova, Olga V. Fedorova, Alexei Y. Bagrov

I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Science, St. Petersburg, Russia, Laboratory of Cardiovascular Science, National Institute on Aging, NIH, Baltimore, MD

TABLE OF CONTENTS

1. ABSTRACT

Dysregulation of the Na/K-ATPase (NKA) in the kidney, cardiovascular system, and peripheral nervous system is believed to contribute to pathogenesis of diabetes mellitus (DM) and its complications. Recently we demonstrated that, in addition to endogenous ouabain (EO), mammalian tissues contain another NKA inhibitor, a bufadienolide marinobufagenin (MBG). *In vitro* MBG, a natriuretic and a vasoconstrictor, acts as a selective inhibitor of alpha-1 NKA, the main isoform of the sodium pump in renal tubules and vascular smooth muscle. To determine whether digitalis-like NKA inhibitors are linked to NKA dysregulation in DM, we studied changes in renal excretion and plasma levels of MBG and EO and the activity of erythrocyte NKA in male Wistar rats with type 1 DM and type 2 DM. Rats with type 1 DM were studied four weeks following a single intraperitoneal injection of 65 mg/kg streptozotocin (STZ)(n = 12), and rats with type 2 DM were studied 10 weeks after intraperitoneal injection of STZ during the neonatal period (n = 12). Renal excretion and plasma levels of EO did not change in rats with both types of DM as compared to that in the control groups. Renal excretion (57.5 ± 9.4 pmol/kg/ 3 hours vs. 12.6 ± 2.1 pmol/kg/ 3 hours; $P < 0.01$) and plasma levels (2.23 ± 0.82 nmol/L vs. 0.29 ± 0.07 nmol/L; $P < 0.01$) of MBG increased, and NKA activity in erythrocytes was inhibited by 50% in rats with type 1 DM as compared to controls. In rats with type 2 DM, plasma levels (1.48 ± 0.09 nmol/L vs. 0.46 ± 0.02 nmol/L; $P < 0.01$) and renal excretion (21.3 ± 3.2 pmol/kg/ 3 hours vs. 13.1 ± 2.1 pmol/kg/ 3 hours) of MBG also became elevated, but less than in the animals with type 1 DM. Accordingly, activity of NKA in erythrocytes from rats with type 2 DM was inhibited by 35%. In vitro treatment of erythrocytes from rats with type 1 and type 2 DM with anti-MBG antibody reversed the DM induced inhibition of the NKA. These results suggest that digitalis-like factors are involved in the pathogenesis of DM, and that MBG, rather than EO, is responsible for DM-induced NKA inhibition.

2. INTRODUCTION

According to the "Concept of Natriuretic Hormone" which evolved from the studies of Dahl, deWardener, Blaustein and others, endogenous digitalis-like sodium pump ligands (SPL) play a role in sodium homeostasis and in the regulation of plasma volume and contribute to pathogenesis of NaCl-sensitive hypertension (1-3). In NaCl-sensitive hypertension, SPL are produced with the primarily adaptive aim of promoting natriuresis via inhibition of the sodium pump in renal tubules. Heightened SPL levels, however, exhibit a maladaptive effect, and contribute to hypertension via inhibition of the Na/K-ATPase in cardiovascular tissues (2). Similar to its receptor sites, isoforms of the alpha-subunit of the Na/K-ATPase, SPLs represent a heterogenic family of compounds which include a cardenolide, endogenous ouabain (EO)(4) and bufadienolides, marinobufagenin (MBG)(5), and bufalin and its analogs (6,7). Likewise, digitalis-like SPL exert differential affinity towards alpha-isoforms of the Na/K-ATPase. Thus, ouabain has high affinity to alpha-2/alpha-3 sodium pump isoforms, while MBG exhibits selectivity towards alpha-1 Na/K-ATPase, an exclusive isoform in the renal tubules and major isoform in the vascular smooth muscle (8,9).

NaCl-sensitivity of the blood pressure is frequently associated with insulin resistance and high NaCl intake was shown to induce insulin signaling in various tissues (10,11). Accordingly, patients with NaCl-sensitive hypertension are prone to development of diabetes mellitus (DM)(12). Patients with DM, in turn, frequently develop NaCl-sensitive volume-dependent hypertension (13). Therefore, one can expect the increased amounts of SPL to be elaborated in the diabetics.

Dysregulation of many cellular regulatory systems, including Na/K-ATPase, are implicated in the pathogenesis of DM and its complications (14-16). Altered expression and functions of the Na/K-ATPase and/or enhanced levels of SPL have been reported in patients with both type 1 and type 2 DM, as well as in rats with streptozotocin induced DM (17-20). However, the nature of SPL(s) implicated in pathogenesis of experimental DM, and whether the inhibition of the Na/K-ATPase observed in the individuals with DM is related to the changes in SPL levels, remains unknown.

The goals of the present study were to compare renal excretion and plasma levels of EO and MBG in rats with streptozotocin (STZ)-induced type1 and type 2 DM, and to find out which SPL is responsible for DM-induced Na/K-ATPase inhibition.

3. MATERIALS AND METHODS

3.1. Induction of DM in rats

The protocol of the study has been approved by the Research Council of Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia in accordance with the rules and regulations of the National Institutes of Health. Two rat DM models were used in our experiments. Male Wistar rats were obtained from the colony of Sechenov Institute of Evolutionary Physiology and Biochemistry (Russian Academy of Science, St. Petersburg). Type 1 DM was induced via a single intraperitoneal injection of 65 mg/kg STZ to five-week-old rats (n = 12)(120-150 grams). Twelve control animals received an intraperitoneal injection of the vehicle, a citrate-buffered saline (pH 5.5). STZ-treated rats which exhibited glucosuria were kept for further study and were sacrificed 4 weeks following STZ administration.

Type 2 (non-insulin-dependent) DM was produced by a single subcutaneous injection of 65 mg/kg STZ to neonatal male Wistar rats (n = 12) as described previously (21). The control rats (n = 12) received a single intraperitoneal injection of a citrate-buffered saline (pH 5.5). The rats were bred in the laboratory and fed ad libitum. Ten weeks following administration of STZ or vehicle, animals were sacrificed. Rats with type 2 DM exhibited moderate elevation of plasma glucose levels and substantial increases in the plasma levels of insulin (Results).

On the last day of experiment, rats with both types of DM were placed in metabolic chambers, and samples of urine were collected for three hours under conditions of water diuresis (22). As reported previously (22), intragastric administration of 5% body weight water evokes a stable diuretic response in rats for three hours. Then, animals were anesthetized with 50 mg/kg ketamine and sacrificed by bleeding from the abdominal aorta. Plasma samples were collected for measurements of MBG, EO, glucose and insulin.

3.2. Na/K-ATPase

Activity of Na/K-ATPase in erythrocytes was determined, as reported previously in detail, in the presence and in absence of rabbit polyclonal anti-MBG antibody at concentration which in vitro blocks the IC_{50} for the inhibition of rat kidney Na/K-ATPase (23). Erythrocytes were preincubated with Tween-20 (0.5%) in sucrose (250 mmol/L) and Tris buffer (20 mmol/L; pH 7.4, 37°C) for 30 minutes, and were incubated for 30 minutes in the medium (mmol/L): Na 100, K 10, $MgCl_2$ 3, EDTA 0.5, Tris 50, ATP 2 (pH 7.4, 37°C) in the final dilution 1:40. The reaction was stopped by the addition of trichloracetic acid to final concentration 7%. Total ATPase activity was measured by the production of inorganic phosphate (P_i), and Na/K-ATPase activity was estimated as the difference between ATPase activity in the presence and in the absence of 5 mmol/L ouabain. Chemicals used were obtained from Sigma Chemicals (St. Louis, MO).

3.3. Immunoassays

The MBG immunoassay was performed as recently described (5,20,23). The assay is based on competition between immobilized antigen (MBG-glycoside-RNAase) and SPL within the sample for a limited amount of binding sites on polyclonal rabbit anti-MBG antibody (aMBG-P) raised against MBG-glycoside-BSA (1:30,000). Secondary europium-labeled goat anti-rabbit antibody was obtained from Perkin-Elmer, Boston, MA. The cross-reactivity of MBG antibody was (%): MBG -

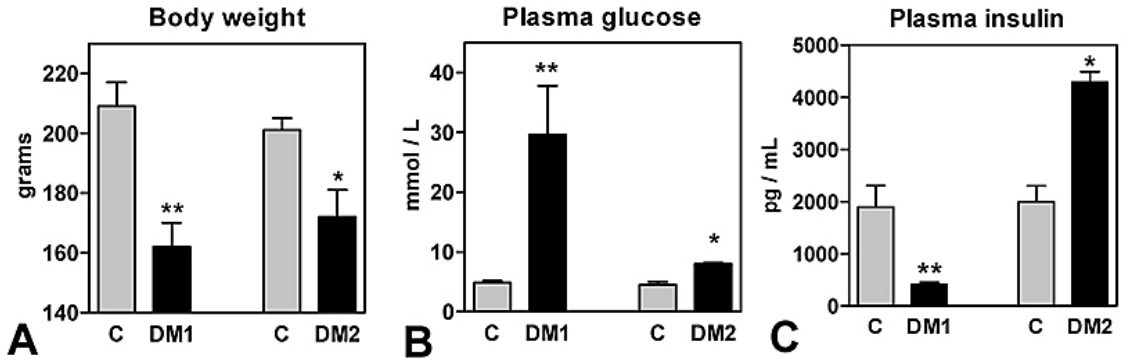

Figure 1. A - Body weights, B - plasma levels of glucose, and C – plasma levels of insulin in controls rats (C) and in rats with STZ-induced type 1 DM (DM1) and type 2 DM (DM2). Each bar represents means ± S.E.M from 12 observations. A: * - P<0.05, ** - P<0.01 vs. control values, two-tailed t-test. B and C: * - P<0.05, ** - P<0.01 vs. control values, repeated one-way ANOVA followed by Newman-Keuls test.

100, ouabain - 0.1, digoxin - 1.0, digitoxin - 3.0, bufalin - 1.0, cinobufagin - 1.0, prednisone - < 0.1, spironolactone - < 0.1, proscillaridin < 1.0, progesterone < 0.1, mixture of bufodienolides from *Bufo marinus* venom except MBG- < 5%. The EO assay was based on a similar principle utilizing an ouabain-ovalbumin conjugate and rabbit ouabain antibody (1:150,000, Chemicon International Inc, Temecula, CA). The cross-reactivity of ouabain antibody is (%): ouabain – 100, digitoxin - 7.4, progesterone < 0.01, 5-beta cholanic acid, prednisone, and canrenoic acid < 0.01, proscillaridin - 0.2, MBG-free mixture of bufodienolides from *Bufo marinus* toad venom - 0.26, bufalin - 0.03, aldosterone - 0.09, MBG- 0.5%. Plasma levels of insulin were determined using Insulin enzyme immunoassay (Crystal Chem Inc., Chicago, IL).

3.4. Statistical analysis

Data are presented as means ± S.E.M. Statistical analyses utilized one-way ANOVA followed by multiple comparisons Newman-Keuls test or by two-tailed t-test, where appropriate (GraphPad Instat and GraphPad Prism, GraphPad Software Inc., San Diego, CA). P values < 0.05 were considered significant.

4. RESULTS

Figure 1 presents body weights and plasma levels of glucose and insulin in the control animals and in rats with both types of DM. Development of both types of DM was associated with a significant reduction in the body weight as compared to that in the control groups. Development of insulin-sensitive type 1 DM was associated with substantially elevated plasma levels of glucose and with a decrease in plasma concentration of insulin. Conversely, following STZ administration to rats during the neonatal period, animals exhibited symptoms of type 2 DM, i.e., moderate but significant elevation of plasma glucose levels and substantial elevation of plasma concentration of immunoreactive insulin.

Figure 2 illustrates changes in renal SPL excretion and plasma levels of MBG and EO in control and diabetic rats. Development of both types of DM was associated with marked increase in renal MBG excretion, although this increase was much more pronounced in the animals with type 1 DM. Thus, in rats with type 1 DM renal MBG excretion increased 4-fold versus the control levels, while in type 2 DM renal excretion of MBG exhibited a 60% increase only. As compared to both control groups, renal excretion of EO did not significantly change in animals with both types of DM (Figure 2 A,B). As demonstrated in Figure 2 C and D, in rats with type 1 DM plasma MBG levels rose 7-fold as compared to that in the control group, while in type 2 DM plasma MBG concentration exhibited a 3-fold increase only. Similar to renal EO excretion, plasma levels of ouabain-like immunoreactivity did not change in rats with both types of DM.

Changes in the activity of erythrocyte Na/K-ATPase are illustrated in Figure 3. Development of both types of DM was accompanied by substantial inhibition of the red blood cell Na/K-ATPase. In rats with type 1 DM in which plasma MBG levels increased 7-fold versus control group, activity of Na/K-ATPase was inhibited by 50% (Figure 3A). At the same time, in rats with type 2 DM, in the presence of a lesser than in DM1 increase in MBG levels, activity of the Na/K-ATPase was inhibited by 30% only (Figure 3B). In rats with both types of DM the in vitro incubation of erythrocytes in the presence of anti-MBG antibody resulted in the restoration of Na/K-ATPase activity to the control levels. At the same time, anti-MBG antibody did not affect the activity of the Na/K-ATPase in the erythrocytes from both control groups (data not shown).

5. DISCUSSION

The main finding of the present experiment is that development of experimental type 1 and type 2 DM in rats is accompanied by enhanced renal excretion and plasma levels of an endogenous bufadienolide Na/K-ATPase inhibitor, MBG, but not that of EO. In rats with both types of DM, heightened MBG levels were accompanied by a substantial inhibition of the Na/K-

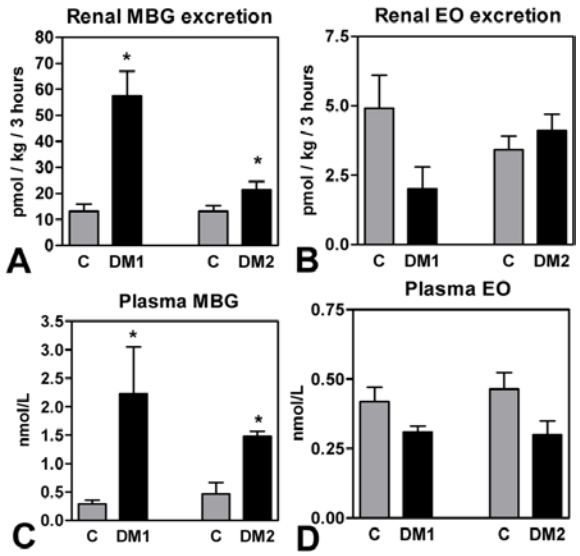

Figure 2. Renal excretion (A and B) and plasma levels (C and D) of MBG and EO in control rats (C) and rats with STZ-induced type 1 DM (DM1) and type 2 DM (DM2). Each bar represents means ± S.E.M from 12 observations. * - P<0.01 vs. control values, two-tailed t-test (A) and Mann-Whitney test (C).

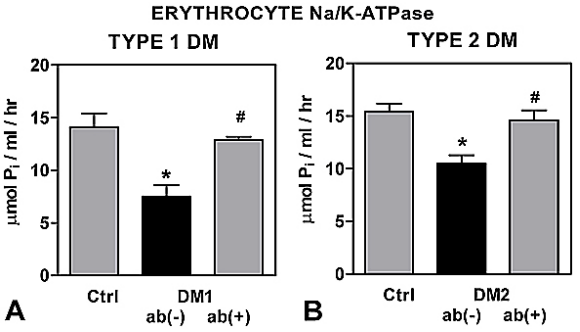

Figure 3. Activity of Na/K-ATPase in erythrocytes from control rats (C) and rats with STZ induced type 1 DM (DM1) and type 2 DM (DM2); ab(-) – in the absence of anti-MBG antibody; ab(+) – in the presence of anti-MBG antibody. By one-way ANOVA followed by Newman-Keuls test: * - P < 0.01 vs. C, # - P< 0.01 vs. ab(-).

TPase in the erythrocytes. Since the *in vitro* pretreatment of erythrocytes from diabetic rats with specific anti-MBG antibody resulted in the restoration of enzyme activity, MBG appears to be one of the factors responsible for DM-induced inhibition of the Na/K-ATPase. The relevance of our present observations to pathogenesis of human DM remains to be proven.

DM is associated with renal sodium retention and diabetics are prone to NaCl-sensitive hypertension (11,12). Previous studies showed that development of STZ-dependent DM in rats is associated with heightened levels of endogenous digoxin-like Na/K-ATPase inhibitors (20,24). Chen, et al. (20) and Martinka, et al. (25) hypothesized that in rats with STZ-induced DM, digoxin-like SPL is elaborated in order to override renal sodium retention and plasma volume expansion. Considering that MBG has high affinity to renal sodium pump (9) and exhibits natriuretic properties *in vivo* (23), we expected that MBG, an inhibitor of renal alpha-1 Na/K-ATPase, rather than EO, would become elevated in the diabetic rats. Since insulin-resistant subjects are prone to hypertension and exhibit greater salt-sensitivity and retain sodium more than insulin-sensitive individuals (12,13), we hypothesized that MBG levels would be higher in rats with type 2 rather than type1 DM. However, in our present experiment, levels of MBG and the degree of Na/K-ATPase inhibition were greater in the animals with type 1 DM as compared to that in rats with type 2 DM. Our present observations are consistent with the results of Dufayet De La Tour, et al. (26) who demonstrated that the activity of red blood cell Na/K-ATPase was lower in type 1 DM than in patients with type 2 DM. Accordingly, in the same study erythrocyte Na/K-ATPase activity exhibited positive linear correlation with plasma levels of C-peptide, one of the markers of insulin resistance (26).

Activation of sodium pump in the renotubular epithelium results in renal sodium retention (27). In type 2 DM renal sodium retention may result from the direct effects of insulin on epithelial sodium transport and on the levels expression of renal Na/K-ATPase (28). Additionally, two other factors implicated in pathogenesis of type 2 DM, leptin, which inhibits the sodium pump (29), and C-peptide, which activates the Na/K-ATPase (30), may be expected to further impair the natriuretic function. The type 2 DM-induced dysregulation of leptin-induced signaling in renotubular epithelium may lead to a further reduction in renal sodium excretion resulting from activation of the sodium pump (29). Thus, in obese Zucker rats, natriuretic activity of leptin is impaired as compared to that in Sprague-Dawley and lean Zucker rats (31). Similarly, dietary-induced obesity in rats was reported to reduce the *in vivo* natriuretic effect of leptin (32). Furthermore, enhanced levels of C-peptide occurring in type 2 DM may contribute further to renal Na/K-ATPase activation and sodium retention (30). Since the above mechanisms are relevant to the pathogenesis of type 2, rather than type 1 DM, one would expect that endogenous levels of SPL would be greater in rats with type 2 DM. Nevertheless, in the present experiment, rats with type 1 DM and low plasma insulin levels exhibited greater increases in MBG and greater Na/K-ATPase inhibition than rats with type 2 DM and high plasma insulin concentration. It may be argued that in the present experiment rats with type 1 DM were studied rather late when they begun to exhibit insulin resistance. However, the fact that rats with type 1 DM exhibited substantial increases in renal MBG excretion within one week following STZ administration (33) argues against such a possibility. Thus, sodium retention is unlikely to be a single stimulus for SPL elaboration in rats with STZ-induced DM.

Although two types of DM, insulin-sensitive and insulin-resistant, exhibit substantial differences in their pathogenesis, hyperglycemia is a common factor for type 1 and type 2 DM. Since in the present experiment, blood

levels of glucose, as the levels of MBG, were higher in rats with type 1 DM as compared to the animals with type 2 DM, a question arises whether elaboration of SPL may be related to regulation of glucose metabolism.

For several decades it has been know that inhibition of the Na/K-ATPase in a live cell is associated with activation of another enzyme, glucose-6phosphate dehydrogenase (G6PD)(34). In several earlier studies of digitalis-like natriuretic hormone, Na/K-ATPase inhibitory activity of putative SPLs was based on the estimation of their ability to stimulate G6PD (35,36). Hyperglycemia is known to trigger the pentose-phosphate pathway of glucose metabolism. The first step of this pathway is catalyzation of glucose-6-phosphate by G6PD (37). Previously, G6PD deficiency has been described in diabetics (38), and subjects deficient in G6PD were reported to abnormally respond to oral glucose tolerance tests (39). Activation of G6PD, on the contrary, was shown to accompany beneficial effects of vanadate treatment in non-obese diabetic mice (40). Since both digitalis and endogenous digitalis-like material were shown to rapidly and reversibly stimulate G6PD (34), regulation of glucose metabolism may become one of the new functions of endogenous digitalis-like factors.

6. ACKNOWLEDGEMENTS

The experiments described in this paper were supported by the Russian Foundation for Fundamental Science (grants 99-04-49529 and 02-04-49337) and by the National Institute on Aging, National Institutes of Health Intramural Research Program.

7. REFERENCES

1. Dahl L.K., K.D. Knudsen & J. Iwai: Humoral transmission of hypertension: Evidence from parabiosis. *Circ Res* 24 Suppl, 21-33 (1969)
2. DeWardener H.E. & E.M. Clarkson: Concept of natriuretic hormone. *Physiol Rev* 65, 658-759 (1985)
3. Blaustein M.P.: Sodium ions, calcium ions, blood pressure regulation and hypertension: A reassessment and a hypothesis. *Am J Physiol* 232, C167-C173 (1977)
4. Ludens J.H., M.A. Clark, K.P. Kolbasa, & J.M. Hamlyn: Digitalis-like factor and ouabain-like compound in plasma of volume-expanded dogs. J Cardiovasc Pharmacol 22 Suppl 2, S38-S41 (1993)
5. Bagrov A.Y., O.V. Fedorova, R.I. Dmitrieva, W.N. Howald, A.P Hunter, E.A. Kuznetsova & V.M. Shpen: Bufodienolide nature of endogenous inhibitor of Na/K ATPase in the urine from patients with acute myocardial infarction. *Hypertension* 31, 1097-1103 (1998)
6. Lichtstein, D., I. Gati, S. Samuelov, D. Berson, Y. Rosenman, L. Landau & J. Deutsch: Identification of digitalis-like compounds in human cataractous lenses. *Eur J Biochem* 216, 261-268 (1993)
7. Hilton P.J., R.W. White, G.A. Lord, G.V. Garner, D.B. Gordon, M.J. Hilton, L.G. Forni, W. McKinnon, F.M. Ismail, M. Keenan, K. Jones & W.E. Morden: An inhibitor of the sodium pump obtained from human placenta. *Lancet* 348, 303-305 (1996)
8. Fedorova O.V. & A.Y. Bagrov: Inhibition of Na/K ATPase from rat aorta by two endogenous Na/K pump inhibitors, ouabain and marinobufagenin. Evidence of interaction with different alpha-subunit isoforms. *Am J Hypertens* 10, 929-935 (1997)
9. Fedorova O.V., E.G. Lakatta & A.Y. Bagrov: Endogenous Na,K pump ligands are differentially regulated during acute NaCl loading of Dahl rats. *Circulation* 102, 3009-3014 (2000)
10. Galletti F, P. Strazzullo, I. Ferrara, G. Annuzzi, A.A. Rivellese, S. Gatto & M. Mancini: NaCl sensitivity of essential hypertensive patients is related to insulin resistance. *J Hypertens* 15, 1485-1491 (1997)
11. Ogihara T., T. Asano & T. Fujita: Contribution of salt intake to insulin resistance associated with hypertension. *Life Sci* 73, 509-523 (2003)
12. Giner V., A. Coca A & A. de la Sierra: Increased insulin resistance in salt sensitive essential hypertension. *J Hum Hypertens* 15, 481-485 (2001)
13. Salomaa V.V., T.E. Strandberg, H. Vanhanen, V. Naukkarinen, S. Sarna & T.A. Miettinen: Glucose tolerance and blood pressure: long term follow up in middle aged men. *BMJ* 302, 493-496 (1991)
14. Ng Y.C., P.H. Tolerico PH & C.B. Book: Alterations in levels of Na(+)-K(+)-ATPase isoforms in heart, skeletal muscle, and kidney of diabetic rats. *Am J Physiol* 265, E243-E251 (1993)
15. Ver A., I. Szanto, T. Banyasz, P. Csermely, E. Vegh & J. Somogyi: Changes in the expression of Na+/K+-ATPase isoenzymes in the left ventricle of diabetic rat hearts: effect of insulin treatment. *Diabetologia* 40, 1255-1162 (1997)
16. Gerbi A, O. Barbey, D. Raccah, T. Coste, I. Jamme, A. Nouvelot, P. Vague & D. Raccah: Alteration of Na,K-ATPase isoenzymes in diabetic cardiomyopathy: effect of dietary supplementation with fish oil (n-3 fatty acids) in rats. *Diabetologia* 40, 496-505 (1997)
17. Clerico A. & O. Giampietro: Is the endogenous digitalis-like factor the link between hypertension and metabolic disorders as diabetes mellitus, obesity and acromegaly? *Clin Physiol Biochem* 8, 153-168 (1990)
18. Straub R.H., C. Hall, B.K. Kramer, R. Elbracht, K.D. Palitzsch, B. Lang & J. Scholmerich: Atrial natriuretic factor and digoxin-like immunoreactive factor in diabetic patients: their interrelation and the influence of the autonomic nervous system. *J Clin Endocrinol Metab* 81, 3385-3389 (1996)
19. Chan J.C., A. Butt, C.A. Ho, C. Cockram & R. Swaminathan: Relation between blood pressure and serum concentration of ouabain-like substance in non-insulin-dependent diabetes mellitus. *Lancet* 351, 266 (1998)
20. Chen S., C. Yuan, D. Clough, J. Schooley, F.J. Haddy & M.B. Pamnani: Role of digitalis-like substance in the hypertension of streptozotocin-induced diabetes in reduced renal mass rats. *Am J Hypertens* 6, 397-406 (1993)
21. Portha B., L. Picon & G. Rosselin: Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. *Diabetologia* 17, 371-377 (1979)
22. Opava Stitzer S. & M. Martinez-Maldonado: Clearence methods in the rat. in *Methods in Pharmacology, Vol. 4B, Renal Pharmacology, Plenum, NY-London* (1978)
23. Fedorova O.V., M.I. Talan, N.I. Agalakova, E.G. Lakatta & Bagrov AY: An endogenous ligand of α-1

sodium pump, marinobufagenin, is a novel mediator of sodium chloride dependent hypertension. *Circulation* 105, 1122-1127 (2002)
24. Chen S., C.M. Yuan, F.J. Haddy & M.B. Pamnani: Effect of administration of insulin on streptozotocin-induced diabetic hypertension in rat. *Hypertension* 23, 1046-1050 (1994)
25. Martinka E., P. Galajada, M. Ochodnicky, B. Lichardus, S. Straka & M. Mokan: Endogenous digoxin-like immunoreactivity and diabetes mellitus: facts and hypotheses. *Med Hypotheses* 49, 271-275 (1997)
26. Dufayet De La Tour D., D. Raccah, M.F. Jannot, T. Coste, C. Rougerie & P. Vague: Erythrocyte Na/K ATPase activity and diabetes: relationship with C-peptide level. *Diabetologia* 41, 1080-1084 (1998)
27. Aperia A., U. Holtback, M.L. Syren, L.B. Svensson, J. Fryckstedt & P. Greengard: Activation/deactivation of renal Na+,K(+)-ATPase: a final common pathway for regulation of natriuresis. *FASEB J* 8, 436-439 (1994)
28. Sweeney G. & A. Klip: Regulation of the Na+/K+-ATPase by insulin: why and how? *Mol Cell Biochem* 182, 121-133 (1998)
29. Sweeney G. & A. Klip: Mechanisms and consequences of Na,K-pump regulation by leptin and insulin. *Cell Mol Biol* 47, 363-372 (2001)
30. Tsimaratos M., F. Roger, D. Chabardes, D. Mordasini, U. Hasler, A. Doucet, P.Y. Martin & E. Feraille: C-peptide stimulates Na+,K+-ATPase activity via PKC alpha in rat medullary thick ascending limb. *Diabetologia* 46, 124-131 (2003) 31. Beltowski J., G. Wojcicka, D Gorny & A. Marciniak: Human leptin administered intraperitoneally stimulates natriuresis and decreases renal medullary Na+, K+-ATPase activity in the rat - impaired effect in dietary-induced obesity. *Med Sci Monit* 8, BR221-BR229 (2002)
32. Villarreal D., G. Reams, R.H. Freeman & A. Taraben: Renal effects of leptin in normotensive, hypertensive, and obese rats. *Am J Physiol* 275, R2056-R20560 (1988)
33. Bagrov A.Y., N.B. Manusova, I.A. Egorova, O.V. Fedorova, J.I. Shapiro, E.G. Lakatta & A.Y. Bagrov: Endogenous digitalis-like factors are linked to sodium pump inhibition in diabetes mellitus. *J Am Soc Nephrol* 14(Suppl S), 612A (2003)
34. de Wardener H.E: From a third factor to a glucose-6-phosphate dehydrogenase stimulator. *Contrib Nephrol* 100, 172-187 (1992)
35. Holland S., J. Millett, J. Alaghband-Zadeh, H. de Wardener, M. Pamnani & F. Haddy: Cytochemically detectable glucose-6-phosphate dehydrogenase-stimulating/Na-K-ATPase-inhibiting activity of plasma and hypothalamus in reduced renal mass hypertension. *Am J Hypertens* 4, 315-20 (1991)
36. MacGregor G.A., S. Fenton, J. Alaghband-Zadeh, N.D. Markandu, J.E. Roulston & H.E. de Wardener: An increase in a circulating inhibitor of Na+,K+-dependent ATPase: a possible link between salt intake and the development of essential hypertension. *Clin Sci (Lond)* 61 Suppl 7, 17s-20s (1981)
37. Gaskin R.S., D. Estwick & R. Peddi: G6PD deficiency: its role in the high prevalence of hypertension and diabetes mellitus. *Ethnicity & Disease* 11, 749-754 (2001)
38. Muggeo M., P. Moghetti, M. Querena, V. Cacciatori, G. Zoppini, M. Zenere, F. Tosi, D. Travia & E. Bonora: Mononuclear leukocytes from obese patients with type II diabetes have reduced activity of hexokinase, 6-phosphofructokinase and glucose-6-phosphate dehydrogenase. *Horm Metab Res* 25, 160-164 (1993)
39. Monte Alegre S, S.T. Saad, E. Delatre & M.J. Saad: Insulin secretion in patients deficient in glucose-6-phosphate dehydrogenase. *Horm Metab Res* 23, 171-173 (1991)
40. Mosseri R., T. Waner, M. Shefi, E. Shafrir & J. Meyerovitch: Gluconeogenesis in non-obese diabetic (NOD) mice: in vivo effects of vandadate treatment on hepatic glucose-6-phoshatase and phosphoenolpyruvate carboxykinase. *Metabolism* 49, 321-532 (2000)

Key Words: Na^+, K^+ Exchanging ATPase, Antagonists, Inhibitors, Diabetes mellitus, Bufadienolides, Marinobufagenin, Review

Send correspondence: Alexei Y. Bagrov, MD, PhD, Laboratory of Cardiovascular Science, National Institute on Aging, Intramural Research Program, NIH. 5600 Nathan Shock Drive, Baltimore, MD 21224, Tel: 410-558-8290, Fax: 410-558-8150; E-mail: BagrovA@grc.nia.nih.gov

http://www.bioscience.org/current/vol10.htm