Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 55 – 61
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Acute effects of digoxin on plasma aldosterone and cortisol in monkeys

Mei-Mei Kau[a,1], Shu-Fen Kan[b], Jiing-Rong Wang[b], Paulus S. Wang[b], Ying-Tung Lau[c], Shyi-Wu Wang[c,*,1]

[a]National Taipei College of Nursing, Taipei 112, Taiwan, Republic of China
[b]Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 112, Taiwan, Republic of China
[c]Department of Physiology and Pharmacology, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan, Republic of China

Received 4 April 2008; accepted 8 August 2008

Abstract

Digoxin, a cardiac glycoside, is used to increase cardiac contractility via inhibition of Na^+/K^+-adenosinetriphosphatase (ATPase) and increase intracellular calcium in congestive heart failure. Inhibitory effects of digoxin have been demonstrated on the biosynthesis of gonadal hormones and adrenal glucocorticoids in rats. However, acute effects of digoxin on levels of adrenal corticosteroid hormones in the primates in vivo are uncertain. Therefore, we test the hypothesis that a single injection of digoxin decreases the secretion of aldosterone and cortisol in monkeys. An intravenous injection of digoxin (1 μg/kg) inhibited basal and adrenocorticotropin (ACTH)- or KCl-stimulated aldosterone release in monkeys. Furthermore, digoxin induced a decrease in ACTH- and KCl-stimulated cortisol release. Administration of digoxin did not alter plasma concentrations of Na^+ and K^+. Ouabain, a selective inhibitor of Na^+/K^+-ATPase, did not affect ACTH- or KCl-stimulated aldosterone and cortisol release. These results revealed that injection of digoxin induced an inhibitory effect on aldosterone and cortisol secretion in monkeys. Because ouabain did not affect levels of plasma aldosterone or cortisol, we suggest that (1) the Na^+/K^+-ATPase pathway may not be involved in the mechanism of action of digoxin on aldosterone or cortisol secretion in monkeys and/or (2) the Na^+/K^+-ATPase is more sensitive to digoxin than to ouabain in monkeys.

1. Background

Digitalis cardiac glycosides, such as digoxin and digitoxin, are clinically used to increase cardiac contractility in congestive heart failure [1]. Cardiac glycosides elicit their pharmacologic mechanisms via the inhibition of the Na^+/K^+-adenosinetriphosphatase (ATPase) and the increase of intracellular Ca^{2+} concentration; and then they enhance the cardiac contractility [2]. The Na^+/K^+-ATPase also serves as a functional receptor for other cardiac glycosides such as ouabain [3]. However, different species have different sensitivities to ouabain because of varied Na^+/K^+-ATPase isoforms [4-6]. For example, human α_1-isoform of Na^+/K^+-ATPase is highly ouabain sensitive, whereas the rodent α_1-isoform is 1000-fold less sensitive to ouabain [4,5].

Extracardiac effects of cardiac glycosides, like steroidogenesis of gonadal and adrenal steroids, had been described in previous studies. Digoxin inhibits the production of testosterone through a decrease in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in rat testicular interstitial cells [7,8]. In addition, digoxin decreased progesterone release by rat granulosa cells involving the inhibition of post-cyclic AMP pathway, cytochrome P450 side-chain cleavage enzyme, and steroidogenic acute regulatory protein functions [9,10]. Wang et al [11] also found that digoxin decreased the release of corticosterone by rat zona fasciculata-reticularis cells involving the inhibition of the activities of adenylyl cyclase, cytochrome P450 side-chain cleavage enzyme, and 11β-hydroxylase, as well as the functions of cyclic AMP and intracellular calcium [11]. These data indicate that digoxin can directly affect signal transduction pathway and inhibit steroidogenesis in steroid-producing cells.

Endogenous digitalis-like factors, such as digoxin-like factor and ouabain-like factor, have been found in human plasma [12], urine [13], adrenal [14], hypothalamus [15,16],

* Corresponding author. Tel.: +886 3 2118800x5253; fax: +886 3 2118700.
E-mail address: swwang@mail.cgu.edu.tw (S.-W. Wang).
[1] These authors contributed equally to this work.

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.08.006

and adrenal cells in culture [17,18]. In addition, elevated plasma endogenous digoxin-like factor or ouabain-like factor has been demonstrated in some clinical or pathologic conditions in humans, such as pregnancy, renal failure, and hypertension [19-21]. Control of Na^+/K^+-ATPase activity is believed to be an underlying mechanism in the pathophysiology of several diseases, including cardiovascular, neurologic, renal, hepatic, psychiatric, and metabolic disorders [22,23]. These results demonstrated that endogenous digitalis-like factors may play a role in the regulation of blood pressure, blood volume, and the pathophysiology of cardiovascular disorders via effects on Na^+/K^+-ATPase activity in different tissues.

Aldosterone, a steroid hormone produced by zona glomerulosa cells of adrenal cortex, increases sodium reabsorption and potassium excretion in renal tubules and plays an important role in the regulation of blood pressure and blood volume. The effect of digoxin or ouabain on aldosterone secretion is conflicting, such as inhibition, stimulation, or no effect [4,24-29]. We have demonstrated inhibitory effects of digoxin or ouabain on the release of aldosterone and cortisol in human adrenocortical cells in vitro [30]; however, acute effects of digoxin on plasma aldosterone and cortisol in primates are still uncertain. The purpose of the present study was to assess the effects of a single injection of digoxin on the levels of plasma aldosterone and cortisol and the possible role of Na^+/K^+-ATPase in digoxin-induced effects in monkeys.

2. Methods

2.1. Animals

To exclude the effects of gonadal steroids on adrenocorticosteroid secretion, we performed the present study with castrated monkeys. Four male and 1 female castrated monkeys (*Macaca cyclopis*) weighing 8.2 ± 1.5 kg were housed individually in temperature-controlled rooms (22°C ± 1°C) with 14 hours of artificial illumination daily (6:00 AM-8:00 PM). They were fed with Purina monkey chow (Purina Mills, St. Louis, MO) twice daily and provided with fresh fruit once daily, whereas tap water was available ad libitum. The use of monkeys for the present study had been approved and authorized by the Council of Agriculture, Executive Yuan, Republic of China.

2.2. Effects of digoxin and ouabain on plasma aldosterone and cortisol in monkeys

Five castrated monkeys were grouped individually. In the morning (10:00 AM), 5 monkeys were injected with saline, digoxin (1 μg/kg), adrenocorticotropin (ACTH) (5 μg/kg), and ACTH combined with either digoxin or ouabain (a selective Na^+/K^+-ATPase inhibitor, 1 μg/kg) via a catheter in the tibial vein, respectively. At the same day (1:00 PM), 1 monkey was infused intravenously with saline for 30 minutes; and then a single injection of saline (ie, saline + saline group) followed. The other 4 groups were saline + digoxin (1 μg/kg), KCl (a stimulator of aldosterone secretion, 1 mEq/kg) + saline, KCl + digoxin, and KCl + ouabain (1 μg/kg), respectively. Experimental treatments in each group were performed alternately on the 5 monkeys.

Blood samples (1 mL each) were collected at 0, 30, 60, 90, 120, and 180 minutes after intravenous injection or infusion. An equal volume of saline was injected immediately after each bleeding. Plasma was separated by centrifugation at 10 000*g* for 1 minute and stored at −20°C. Plasma was extracted with diethyl ether (10-fold the volume). Levels of aldosterone and cortisol in plasma extracts were measured by radioimmunoassay (RIA).

A flame photometer (EFOX 5053; Eppendorf, Hamburg, Germany) was used to determine the levels of plasma Na^+ and K^+.

2.3. Hormone RIAs

The concentration of aldosterone in extracted plasma was measured by RIA as previously described [31]. The antialdosterone serum no. 088 was provided by the National Institutes of Health (Bethesda, MD). The sensitivity of aldosterone RIA was 4 pg per assay tube. The intra- and interassay coefficients of variation were 7.4% (n = 5) and 7.8% (n = 5), respectively.

Levels of plasma cortisol were determined by RIA. An antiserum to cortisol was generated by immunizing rabbits with 4-pregnen-11β,17,21-triol-3,20-dione 3-CMO:bovine serum albumin (BSA) conjugate (Steraloids, Wilton, NH). With this antiserum no. PW-212, an RIA was established for the measurement of cortisol in plasma samples. In this RIA system, a known amount of unlabeled cortisol or an aliquot of plasma extract was adjusted to a total volume of 0.3 mL by a buffer solution (1% BSA-borate buffer, pH 7.8). Each sample was incubated with 0.1 mL cortisol antiserum (1:20 000 dilution) diluted with 1% BSA-borate buffer and 0.1 mL ^{3}H-cortisol (approximately 8000 cpm; Amersham, Buckinghamshire, United Kingdom) at 4°C for 24 hours. Duplicate standard curves with 5 points ranging from 10 to 2500 pg of cortisol were included in each assay. An adequate amount (0.2 mL) of 0.5% dextran-coated charcoal (Sigma, St Louis, MO) was then added before a further incubation in an ice bath for 15 minutes. At the end of the incubation, the assay tubes were centrifuged at 1000*g* for 15 minutes. The supernatant was mixed with 3 mL liquid scintillation fluid (Ready Safe; Beckman, Fullerton, CA) before the radioactivity was counted in an automatic β counter (Wallac 1409; Pharmacia, Turku, Finland). The maximum binding of ^{3}H-cortisol with anticortisol antiserum was 27%. The sensitivity of cortisol RIA was 10 pg per assay tube. The cross-reactions were 4.9% with corticosterone, 4.0% with testosterone, 0.9% with progesterone, and less than 0.01% with aldosterone, androstenediol, estrone, 17α-hydroxyprogesterone, 17β-estradiol, β-estradiol, cholesterol, 4-androstene-3,17-dione, pregnenolone, 17α-estradiol, estriol, and androstanolone.

The intra- and interassay coefficients of variation were 9.8% (n = 5) and 14.6% (n = 3), respectively.

2.4. Statistical analysis

The data were expressed as mean ± SEM. Homogeneity of variance and analysis of data were performed by analysis of variance of repeated measures [32]. All the statistics was calculated by SPSS, version 11.0 (SPSS, Chicago, IL); and the difference was considered significant if P was less than .05.

3. Results

3.1. Effects of digoxin on plasma aldosterone

As shown in Fig. 1, intravenous administration of saline did not affect the level of plasma aldosterone. A single injection of digoxin (1 μg/kg) reduced aldosterone release, and this response was different from the saline group at 30 and 60 minutes after injection ($P < .05$ and $P < .01$). Plasma level of aldosterone recovered toward basal levels at 90 minutes after digoxin injection. At 30 minutes after ACTH (5 μg/kg) injection, the level of plasma aldosterone increased from 123 ± 7 to 513 ± 10 pg/mL (420% ± 25%) and then gradually returned to basal levels. A single injection of ACTH combined with digoxin increased aldosterone release from 130 ± 7 to 316 ± 9 pg/mL (246% ± 16%). Markedly, digoxin blunted the ACTH-stimulated aldosterone secretion ($P < .01$). However, ouabain (a selective Na^+/K^+-ATPase inhibitor) did not affect ($P > .05$) the ACTH-stimulated aldosterone secretion (Fig. 1, lower panel).

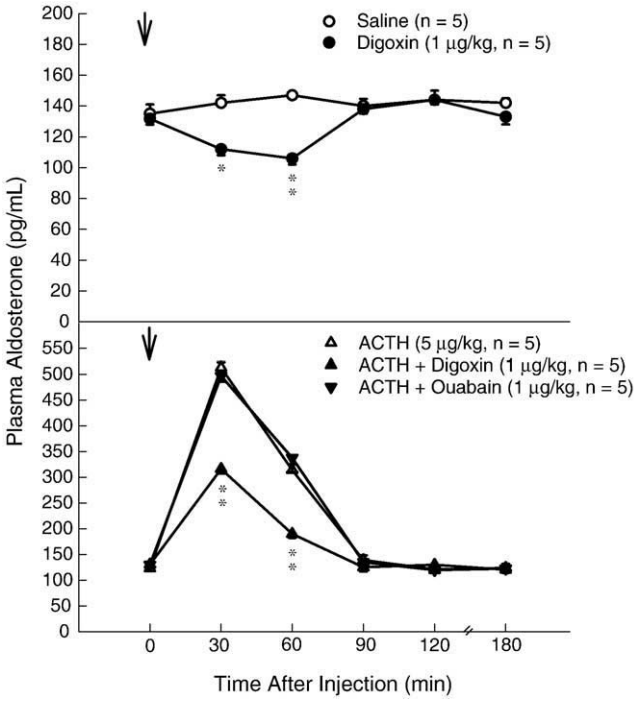

Fig. 1. Effects of digoxin or ouabain on the basal and ACTH-stimulated aldosterone secretion in monkeys. Each value represents mean ± SEM. *$P <$ .05, **$P < .01$ as compared with the group without digoxin injection at the same time point, respectively. The arrow (↓) represents a single intravenous injection of saline, digoxin, ACTH, ACTH combined with digoxin, or ACTH combined with ouabain, respectively.

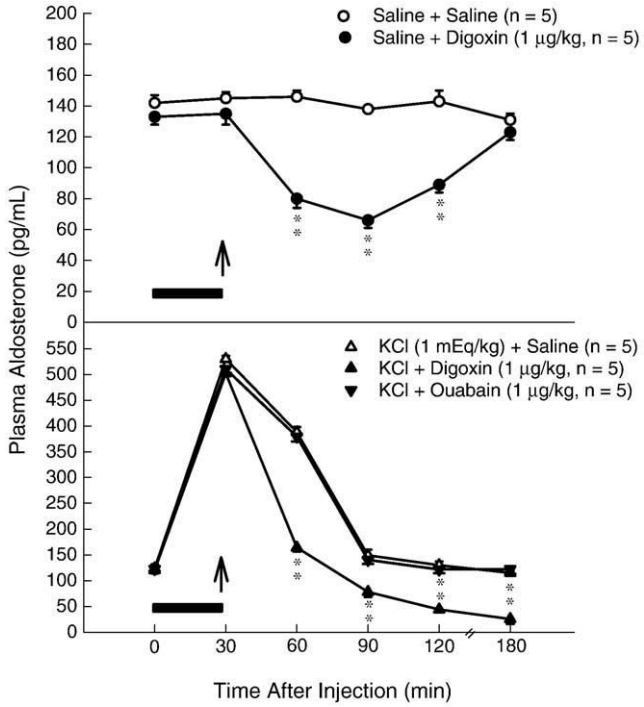

Fig. 2. Effects of digoxin or ouabain on the basal and KCl-stimulated aldosterone secretion in monkeys. Each value represents mean ± SEM. **$P < .01$ as compared with the group without digoxin injection at the same time point. The horizontal bar (▬) represents saline or KCl infusion for 30 minutes. The arrow (↑) represents a single intravenous injection of saline, digoxin, or ouabain, respectively.

In the groups of saline + saline and saline + digoxin, intravenous infusion of saline for 30 minutes did not alter levels of plasma aldosterone (Fig. 2, upper panel). Moreover, there was no effect of a single injection with saline on aldosterone secretion in the saline + saline group (Fig. 2, upper panel). A single injection of digoxin decreased the level of plasma aldosterone, and there was difference between the groups of saline + saline and saline + digoxin ($P < .01$). Two and a half hours after digoxin injection, the levels of aldosterone returned to basal levels (Fig. 2, upper panel). In the KCl + saline group, KCl infusion for 30 minutes increased plasma K^+ (from 3.5 ± 0.1 to 6.0 ± 0.2 mEq/L). After K^+ infusion and after saline injection, plasma K^+ levels returned to basal levels (4.0 ± 0.2 mEq/L). Potassium chloride (1 mEq/kg) infusion for 30 minutes increased levels of aldosterone in both the KCl + saline and KCl + digoxin groups (Fig. 2, lower panel). In the KCl + saline group, levels of aldosterone gradually decreased after termination of KCl infusion and saline injection. In the KCl + digoxin group, a single injection of digoxin induced a more rapid attenuation in the level of plasma aldosterone than it

did in the KCl + saline group ($P < .01$). In addition, there was no difference ($P > .05$) in aldosterone release between the group of KCl + saline and the group of KCl + ouabain.

3.2. Effects of digoxin on plasma cortisol

It has been well known that cortisol is a major glucocorticoid for primates that regulates body's various responses to stress. As shown in Figs. 3 and 4, saline injection did not alter the levels of plasma cortisol. These results showed that our treatment should not generate a stress response on monkeys. In Fig. 3 (upper panel), there was no difference ($P > .05$) in cortisol release between the groups of saline and digoxin. In the ACTH-injected group, the level of plasma cortisol gradually increased from 30 to 120 minutes after ACTH injection; and it returned to basal levels at 180 minutes (Fig. 3, lower panel). Compared with the ACTH-injected group (Fig. 3, lower panel, $P < .01$), ACTH-increased cortisol was attenuated in the ACTH + digoxin treatment group. Ouabain injection did not alter the effects of ACTH on cortisol release (Fig. 3, lower panel). There was no difference ($P > .05$) in cortisol release between the group of saline + saline and the group of saline + digoxin (Fig. 4, upper panel). After KCl infusion for 30 minutes and a following single injection of saline, the level of plasma cortisol gradually increased (Fig. 4, lower panel). In the KCl + digoxin group, digoxin injection almost abolished the KCl-stimulated cortisol release when compared with the KCl + saline group ($P < .01$ and $P < .05$). Similarly, ouabain did not affect the KCl-produced increase in the level of plasma cortisol.

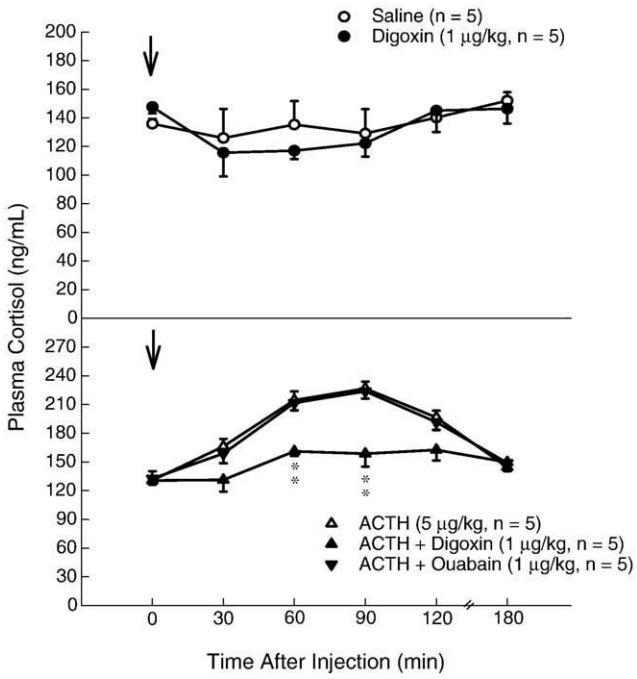

Fig. 3. Effects of digoxin or ouabain on the basal and ACTH-stimulated cortisol secretion in monkeys. Each value represents mean ± SEM. $^{**}P < .01$ as compared with the group without digoxin injection at the same time point. The arrow (↓) represents a single intravenous injection of saline, digoxin, ACTH, ACTH combined with digoxin, or ACTH combined with ouabain, respectively.

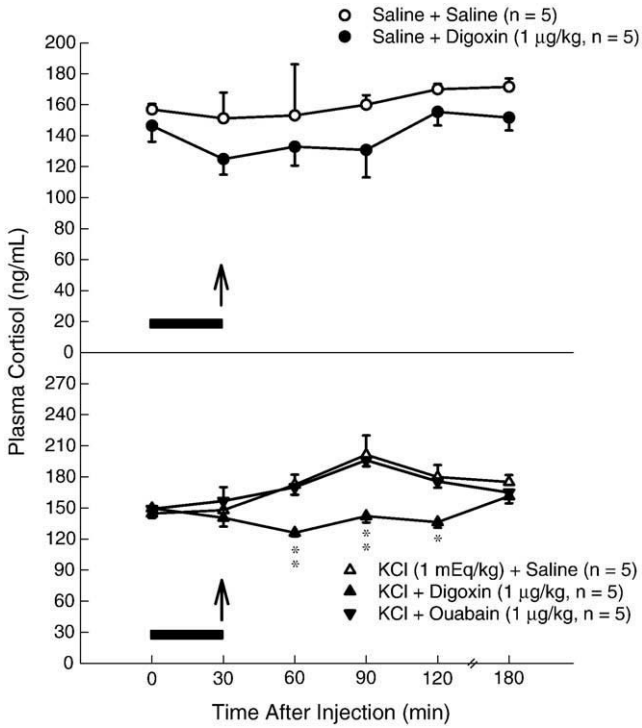

Fig. 4. Effects of digoxin or ouabain on the basal and KCl-stimulated cortisol secretion in monkeys. Each value represents mean ± SEM. $^{*}P < .05$, $^{**}P < .01$ as compared with the group without digoxin injection at the same time point. The horizontal bar (▬) represents saline or KCl infusion for 30 minutes. The arrow (↑) represents a single intravenous injection of saline, digoxin, or ouabain, respectively.

3.3. Effects of digoxin on plasma Na^+ and K^+

The effects of digoxin on the levels of plasma Na^+ and K^+ are illustrated in Table 1. Neither Na^+ nor K^+ concentration in monkey plasma was altered by digoxin.

4. Discussion

In the present study, a single injection of digoxin induced a significant inhibition in basal and ACTH- or KCl-

Table 1
Effects of digoxin (1 μg/kg) on the levels of plasma Na^+ or K^+ in monkeys

	Before injection	After injection (min)		
	0	30	60	90
Plasma [Na^+] (mEq/L, n = 5)	143.0 ± 1.3	143.8 ± 1.2	144.7 ± 1.4	144.5 ± 1.4
Plasma [K^+] (mEq/L, n = 5)	4.4 ± 0.1	4.5 ± 0.2	4.3 ± 0.3	4.1 ± 0.3

stimulated aldosterone secretion in monkeys. Digoxin also significantly reduced ACTH- and KCl-stimulated cortisol release, whereas there was no highly significant effect on basal release of cortisol in monkeys. These effects of digoxin on cortisol release in monkeys are similar to the previous study showing an inhibitory effect of digoxin on corticosterone release in rats [11]. Our previous in vitro studies had demonstrated that digoxin directly inhibits glucocorticoids or aldosterone secretion through attenuated steroidogenesis in rat [11] or human [30] adrenocortical cells. We still cannot exclude the possibility that the inhibitory effect of digoxin on cortisol or aldosterone release is indirectly induced by the changes of ACTH or plasma renin activity because ACTH or renin is involved in the regulation of cortisol or aldosterone secretion.

It has been well known that digitalis glycosides produce a positive inotropic effect on cardiac muscle through an inhibition of the Na^+/K^+-ATPase and an elevation of intracellular calcium concentration [2]. Previous studies demonstrated that digoxin can directly inhibit the biosynthesis of testosterone, progesterone, or corticosterone by testicular interstitial cells, granulosa cells, or adrenal zona fasciculata-reticularis cells in rats, whereas ouabain (a selective inhibitor of Na^+/K^+-ATPase) cannot produce any effects [7,10,11]. In the same manner, ouabain did not affect aldosterone and cortisol secretion of monkeys in the present experiment. On the contrary, ouabain showed an inhibitory effect on aldosterone secretion in human or bovine adrenocortical cells [30,33]. The reasons for these contradictory results among different species may be due to different sensitivity to ouabain through different Na^+/K^+-ATPase isoforms [4-6]. For example, human α_1-isoform of Na^+/K^+-ATPase is 1000-fold more sensitive to ouabain than rodent α_1-isoform of Na^+/K^+-ATPase [4,5]. Another explanation is that Na^+/K^+-ATPase probably is not involved in the effects of digoxin on aldosterone secretion. Finally, digoxin is much more lipophilic than ouabain; and therefore, it is more difficult for ouabain to cross the cell membrane by diffusion in a short term.

It is well documented that digoxin has a narrow therapeutic window and that the therapeutic range for digoxin in serum is approximately 0.5 to 2.5 ng/mL ($\approx 0.6 \sim 3.2 \times 10^{-9}$ mol/L) [34]. The clinical adverse effects associated with high concentrations of cardiac glycosides in serum include gastrointestinal irritation, hyperkalemia, atrioventricular block, and ventricular dysrhythmia [35]. Struthers et al [36] have demonstrated that a moderate dose of digoxin (50 μg/kg) had no effect on plasma K^+ (4.7 ± 0.2 to 4.5 ± 0.2 mEq/L). Although we have not determined the plasma level of digoxin, the dose of digoxin (1 μg/kg) in our present study was much less than that in the study of Struthers et al [36]. Therefore, we predict that the dose of 1 μg/kg digoxin should not produce the problem of being over the therapeutic range. In the present study, digoxin administration did not change the level of plasma K^+. These results indicate that the decreased aldosterone secretion by digoxin is independent of the change of plasma K^+.

Ludens et al [14] have demonstrated that the adrenal gland is the major source of circulating ouabain. Endogenous ouabain or digoxin was identified in the medium of cultured cells from bovine adrenocortical cells [18] and human adrenocortical cells [30,37]. Previous reports showed that Ang II stimulated the secretions of aldosterone and endogenous ouabain from bovine adrenocortical cells via different AT_1 and AT_2 receptor subtypes [38-40]. The physiologic significance of Ang II on endogenous ouabain release in adrenocortical cells is unknown and remains to be elucidated. Previous [24] and present studies demonstrated that digoxin significantly inhibited aldosterone secretion. These observations raise the possibility of the existence of an autocrine/paracrine regulation between adrenocortical steroidogenesis and endogenous digoxin- or ouabain-like compounds.

It has been shown that aldosterone plays an important role in the pathophysiology of heart failure [41,42]. Aldosterone, aside from sodium retention and potassium loss, promotes myocardial and vascular fibrosis [43,44] as well as vascular damage [45]. Pitt et al [46] have reported that the aldosterone-receptor blocker spironolactone significantly reduced the risk of both morbidity and mortality among patients with heart failure. Although the exact mechanism is unclear, it can be proposed that spironolactone may prevent myocardial fibrosis by blocking the effect of aldosterone on the formation of collagen [47,48] and reduce the risk of sudden death of cardiac causes. These results provide valuable information about the pharmacologic mechanisms of digoxin, which may play a protective effect through a decreased aldosterone release in patients of heart failure except increase myocardial contractility.

The present study showed that injection of digoxin induced an inhibitory effect on aldosterone and cortisol secretion in monkeys. Many previous results demonstrated that endogenous digitalis-like factors may play a role in the regulation of blood pressure, blood volume, and the pathophysiology of cardiovascular disorders. Although the detailed mechanism is unclear, our results provide an explanation for the role of endogenous digitalis-like factors in the regulation of cardiovascular system. In our present and previous studies, ouabain (a selective inhibitor of Na^+/K^+-ATPase) caused contradictory results among different species. Therefore, we believe that it will raise an interesting and significant topic that different species may possess different sensitivities to ouabain through different Na^+/K^+-ATPase isoforms.

Acknowledgment

The authors thank Ms HL Chao for her experimental assistance. This study was supported by the grant from the National Science Council (NSC94-2621-Z-010-003), Taiwan, Republic of China. The antialdosterone antiserum no.

088 was kindly supplied by the National Institutes of Health (Bethesda, MD).

References

[1] Iisalo E. Clinical pharmacokinetics of digoxin. Clin Pharmacokinet 1977;2:1-16.

[2] Hougen TJ, Smith TW. Inhibition of myocardial monovalent cation active transport by subtoxic doses of ouabain in the dog. Circ Res 1978;42:856-63.

[3] Lingrel JB, Kuntzweiler T. Na^+, K^+-ATPase. J Biol Chem 1994;269: 19659-62.

[4] Antonipillai I, Schick K, Horton R. Ouabain is a potent inhibitor of aldosterone secretion and angiotensin action in the human adrenal. J Clin Endocrinol Metab 1996;81:2335-7.

[5] Hansen O. No evidence for a role in signal-transduction of Na+/K+-ATPase interaction with putative endogenous ouabain. Eur J Biochem 2003;270:1916-9.

[6] Sweadner KJ. Isozymes of the Na+/K+-ATPase. Biochim Biophys Acta 1989;988:185-220.

[7] Lin H, Wang SW, Tsai SC, Chen JJ, Chiao YC, Lu CC, et al. Inhibitory effect of digoxin on testosterone secretion through mechanisms involving decreases of cyclic AMP production and cytochrome P450scc activity in rat testicular interstitial cells. Br J Pharmacol 1998;125:1635-40.

[8] Wang SW, Lin H, Hwang JJ, Wang PS. Inhibition of testosterone secretion by digoxin in rat testicular interstitial cells. J Cell Biochem 1999;74:74-80.

[9] Chen JJ, Chien EJ, Wang PS. Progesterone attenuates the inhibitory effects of cardiotonic digitalis on pregnenolone production in rat luteal cells. J Cell Biochem 2002;86:107-17.

[10] Chen JJ, Wang PS, Chien EJ, Wang SW. Direct inhibitory effect of digitalis on progesterone release from rat granulosa cells. Br J Pharmacol 2001;132:1761-8.

[11] Wang SW, Pu HF, Kan SF, Tseng CI, Lo MJ, Wang SW. Inhibitory effects of digoxin and digitoxin on corticosterone production in rat zona fasciculata-reticularis cells. Br J Pharmacol 2004;142:1123-30.

[12] Hamlyn JM, Blaustein MP, Bova S, DuCharme DW, Harris DW, Mandel F, et al. Identification and characterization of an ouabain-like compound from human plasma. Proc Natl Acad Sci U S A 1991;88: 6259-63.

[13] De Angelis C, Riscazzi M, Salvini R, Piccoli A, Ferri C, Santucci A. Isolation and characterization of a digoxin-like immunoreactive substance from human urine by affinity chromatography. Clin Chem 1997;43:1416-20.

[14] Ludens JH, Clark MA, Robinson FG, DuCharme DW. Rat adrenal cortex is a source of a circulating ouabainlike compound. Hypertension 1992;19:721-4.

[15] Li S, Eim C, Kirch U, Lang RE, Schoner W. Bovine adrenals and hypothalamus are a major source of proscillaridin A- and ouabain-immunoreactivities. Life Sci 1998;62:1023-33.

[16] Tymiak AA, Norman JA, Bolgar M, DiDonato GC, Lee H, Parker WL, et al. Physicochemical characterization of an ouabain isomer isolated from bovine hypothalamus. Proc Natl Acad Sci U S A 1993;90: 8189-93.

[17] Doris PA, Hayward-Lester A, Bourne D, Stocco DM. Ouabain production by cultured adrenal cells. Endocrinology 1996;137: 533-9.

[18] Laredo J, Hamilton BP, Hamlyn JM. Ouabain is secreted by bovine adrenocortical cells. Endocrinology 1994;135:794-7.

[19] Graves SW, Brown B, Valdes Jr R. An endogenous digoxin-like substance in patients with renal impairment. Ann Intern Med 1983;99: 604-8.

[20] Rossi G, Manunta P, Hamlyn JM, Pavan E, De Toni R, Semplicini A, et al. Immunoreactive endogenous ouabain in primary aldosteronism and essential hypertension: relationship with plasma renin, aldosterone and blood pressure levels. J Hypertens 1995;13:1181-91.

[21] Valdes Jr R, Graves SW. Protein binding of endogenous digoxin-immunoactive factors in human serum and its variation with clinical condition. J Clin Endocrinol Metab 1985;60:1135-43.

[22] Doris PA, Bagrov AY. Endogenous sodium pump inhibitors and blood pressure regulation: an update on recent progress. Proc Soc Exp Biol Med 1998;218:156-67.

[23] Hollenberg NK, Graves SW. Endogenous sodium pump inhibition: current status and therapeutic opportunities. Prog Drug Res 1996;46:9-42.

[24] Braley LM, Williams GH. The effects of ouabain on steroid production by rat adrenal cells stimulated by angiotensin II, alpha 1-24 adrenocorticotropin, and potassium. Endocrinology 1978;103: 1997-2005.

[25] Covit AB, Schaer GL, Sealey JE, Laragh JH, Cody RJ. Suppression of the renin-angiotensin system by intravenous digoxin in chronic congestive heart failure. Am J Med 1983;75:445-7.

[26] Guthrie Jr GP. Effects of digoxin on responsiveness to the pressure actions of angiotensin and norepinephrine in man. J Clin Endocrinol Metab 1984;58:76-80.

[27] Hajnóczky G, Csordás G, Hunyady L, Kalapos MP, Balla T, Enyedi P, et al. Angiotensin-II inhibits Na^+/K^+ pump in rat adrenal glomerulosa cells: possible contribution to stimulation of aldosterone production. Endocrinology 1992;130:1637-44.

[28] Neri G, De Toni R, Tortorella C, Rebuffat P, Bova S, Cargnelli G, et al. Ouabain chronic infusion enhances the growth and steroidogenic capacity of rat adrenal zona glomerulosa: the possible involvement of the endothelin system. Int J Mol Med 2006;18:315-9.

[29] Pistorello M, Cimolato M, Pedini F, Piovan D, Boscaro M, Fallo F. Effect of digoxin on the in vitro secretion of renin and angiotensin II/III immunoreactivity by the human adrenal gland. Acta Endocrinol 1992; 127:210-4.

[30] Kau MM, Kan SF, Wang JR, Wang PS. Inhibitory effects of digoxin and ouabain on aldosterone synthesis in human adrenocortical NCI-H295 cells. J Cell Physiol 2005;205:393-401.

[31] Kau MM, Chang LL, Kan SF, Ho LT, Wang PS. Stimulatory effects of hyperprolactinemia on aldosterone secretion in ovariectomized rats. J Investig Med 2002;50:101-9.

[32] Steel RGD, Torrie JH. Principles and procedures of statistics. New York: McGraw-Hill; 1960.

[33] Elliott ME, Hadjokas NE, Goodfriend TL. Effects of ouabain and potassium on protein synthesis and angiotensin-stimulated aldosterone synthesis in bovine adrenal glomerulosa cells. Endocrinology 1986; 118:1469-75.

[34] Clark WG, Brater DC, Johnson AR. Digitalis. Goth's medical pharmacology. St. Louis, (Mo): Mosby Year Book; 1992.

[35] Valdes Jr R, Jortani SA, Gheorghiade M. Standards of laboratory practice: cardiac drug monitoring. Clin Chem 1998;44:1096-109.

[36] Struthers AD, Davies DL, Harland D, Price JS, Brown RA, Quigley C, et al. Adrenaline causes potassium influx in skeletal muscle and potassium efflux in cardiac muscle in rats: the role of Na/K ATPase. Life Sci 1987;40:101-8.

[37] El-Masri MA, Clark BJ, Qazzaz HM, Valdes Jr R. Human adrenal cells in culture produce both ouabain-like and dihydroouabain-like factors. Clin Chem 2002;48:1720-30.

[38] Laredo J, Shah JR, Lu ZR, Hamilton BP, Hamlyn JM. Angiotensin II stimulates secretion of endogenous ouabain from bovine adrenocortical cells via angiotensin type 2 receptors. Hypertension 1997;29: 401-7.

[39] Shah JR, Laredo J, Hamilton BP, Hamlyn JM. Different signaling pathways mediate stimulated secretions of endogenous ouabain and aldosterone from bovine adrenocortical cells. Hypertension 1998;31: 463-8.

[40] Shah JR, Laredo J, Hamilton BP, Hamlyn JM. Effects of angiotensin II on sodium potassium pumps, endogenous ouabain, and aldosterone in bovine zona glomerulosa cells. Hypertension 1999; 33:373-7.

[41] Barr CS, Lang CC, Hanson J, Arnott M, Kennedy N, Struthers AD. Effects of adding spironolactone to an angiotensin-converting enzyme inhibitor in chronic congestive heart failure secondary to coronary artery disease. Am J Cardiol 1995;76:1259-65.

[42] Swedberg K, Eneroth P, Kjekshus J, Wilhelmsen L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. Circulation 1990;82:1730-6.

[43] Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT. Remodeling of the rat right and left ventricles in experimental hypertension. Circ Res 1990;67:1355-64.

[44] Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991;83:1849-65.

[45] Rocha R, Chander PN, Khanna K, Zuckerman A, Stier Jr CT. Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. Hypertension 1998;31:451-8.

[46] Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. N Engl J Med 1999;341:709-17.

[47] Brilla CG, Matsubara LS, Weber KT. Anti-aldosterone treatment and the prevention of myocardial fibrosis in primary and secondary hyperaldosteronism. J Mol Cell Cardiol 1993;25:563-75.

[48] MacFadyen RJ, Barr CS, Struthers AD. Aldosterone blockade reduces vascular collagen turnover, improves heart rate variability and reduces early morning rise in heart rate in heart failure patients. Cardiovasc Res 1997;35:30-4.