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Effects of Digoxin on the Vascular Reactivity to Infused Norepinephrine in Normotensive Subjects

Hideaki FUJII

The First Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

(Director: Prof. Goro KAJIYAMA)

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ABSTRACT

In order to clarify the role of a circulating digitalis-like substance in the pathogenesis of essential hypertension, short (intravenous) and long term (oral administration) effects of digoxin on the vascular reactivity to infused norepinephrine (NE) at a constant rate (0.22 μg/kg/min) were studied in normotensive male volunteers.

In 14 subjects 90 min after intravenous administration of digoxin, basal intraerythrocytic sodium concentration (ENa) before NE infusion was significantly increased due to inhibition of Na^+, K^+ ATPase activity in cell membrane by digoxin indicated as erythrocyte ouabain sensitive efflux rate constant (ERCos), and basal plasma NE concentration (PNE) was significantly increased. Basal hemodynamic parameters except for heart rate (HR) remained unchanged, but increases in mean blood pressure and total peripheral resistance during NE infusion (ΔMBP and ΔTPR) were significantly augmented. Increase in PNE during NE infusion (ΔPNE) tended to decrease. Intravenous administration of digoxin significantly augmented ΔMBP/ΔPNE and ΔTPR/ΔPNE as the parameters for the pressor response and vascular reactivity to NE from 3.81 ± 1.64 $mmHg \cdot ml \cdot ng^{-1}$ to 4.85 ± 2.12 $mmHg \cdot ml \cdot ng^{-1}$ and from 121 ± 75 $dyne \cdot sec \cdot cm^{-2} \cdot ng^{-1}$ to 147 ± 61 $dyne \cdot sec \cdot cm^{-2} \cdot ng^{-1}$, respectively.

In 14 subjects after 6 days oral administration of digoxin as well as intravenous administration of digoxin, basal ERCos was significantly inhibited and basal ENa was significantly increased. Basal PNE was unchanged, but ΔPNE was significantly decreased. Oral administration of digoxin also significantly augmented the pressor response and vascular reactivity to NE from 5.14 ± 1.70 $mmHg \cdot ml \cdot ng^{-1}$ to 6,25 ± 2.00 $mmHg \cdot ml \cdot ng^{-1}$ and from 130 ± 65 $dyne \cdot sec \cdot cm^{-2} \cdot ng^{-1}$ to 176 ± 53 $dyne \cdot sec \cdot cm^{-2} \cdot ng^{-1}$, respectively.

These findings suggest that the augmented pressor response to NE is caused by the enhanced vascular reactivity to NE through inhibition of Na^+, K^+ ATPase activity in cell membrane by digoxin. Thus, a circulating digitalis-like substance may play an important role in the pathogenesis of essential hypertension.

Since 1952 when Tobian and Binion[41] first reported that the intracellular concentration of sodium in the renal arteries obtained on autopsy from hypertensive patients was raised, considerable interest has been focussed on abnormalities in sodium transport across cell membrane in the pathogenesis of essential hypertension. Abnormalities of sodium transport have been repeatedly demonstrated in erythrocytes[1,17,29,37,42], leucocytes[13,35] or lympho-

cytes[3] of patients with essential hypertension. A reduced activity of sodium pump has been also demonstrated in the vascular smooth muscle cells of animals with volume expanded hypertension[24]. These abnormalities have been explained by a hypothesis that hypertensive patients have an inherited variability in the ability of the kidney to eliminate excessive sodium. The difficulty in eliminating sodium stimulates the hypothalamus to increase the secretion of a circulating digitalis-like substance into the plasma[10]. A raised intracellular sodium through inhibition of Na^+, K^+ ATPase activity in cell membrane by a circulating digitalis-like substance results in a raised intracellular calcium, thus increasing the vascular reactivity to pressor substances[4].

Many investigators have also demonstrated that the augmented vascular reactivity to vasoactive substances plays a primary role in the development and continuation of hypertension[12,33,43]. Although a number of factors have been postulated to explain the altered vascular response to vasoactive substances in essential hypertension[16,40], changes in intracellular sodium and calcium concentration have been assumed to be one of the basic factors in augmented response to vasoactive substances[4].

Cardiac glycosides increase the force of myocardial contraction and this inotropic effect is believed to be a consequence of inhibition of the membrane Na^+, K^+ ATPase[2]. Previous reports have demonstrated that cardiac glycosides have a potentiating effect on vasoconstrictors *in vitro*[15,28] probably in part due to intracellular accumulation of calcium ions after Na^+, K^+ ATPase inhibition, as occurs in myocardium.

In an attempt to clarify the role of a circulating digitalis-like substance in the pathogenesis of essential hypertension, in the present study the effects of digoxin on the vascular reactivity to norepinephrine (NE) were examined in normotensive subjects.

MATERIALS AND METHODS

Materials

dl-norepinephrine was obtained from Sankyo Co., (Tokyo, Japan), digoxin from Chugai Co. (Tokyo, Japan) and methyldigoxin from Yamanouchi Co. (Tokyo, Japan). ^{125}I-angiotensin I radioimmunoassay (RIA) kit and ^{3}H-aldosterone RIA kit were purchased from CEA-IRE-SORIN (Paris, France) and ^{125}I-tyrosyl-digoxin RIA kit from Dinabot Co. (Tokyo, Japan).

Subjects and Study Protocol

In this study, 32 male volunteers aged 24 to 34 years (mean age of 27 ± 3 years; mean ± standard deviation (SD)) were used as subjects. These subjects were requested to be on a regular diet containing 10 to 13 g of salt per day during the study, and 24-hr urinary sodium excretion (UNaV) was measured for 3 days during the study. Adequacy of urine collection was checked by daily creatinine determinations. No medication was provided. The study protocol was explained to each subjects and informed consent was individually obtained.

Fig. 1 illustrates the study protocol. In the preliminary study to evaluate the effects of repetitive NE infusion or placebo on the parameters before NE infusion and their changes during NE infusion, NE infusion test was performed in 8 men (mean age of 29 ± 3 years) or in 6 men (mean age of 30 ± 3 years) before and after the injection of 5% glucose solution or oral administration of placebo. The effects of intravenous digoxin on the vascular reactivity to constantly infused NE were studied in 14 men (mean age of 27 ± 2 years) before and 90 min after intravenous administration of 0.25 mg of digoxin. The effects of oral digoxin on the vascular reactivity to NE were also studied in 14 men (mean age of 27 ± 2 years) before and after 6 days oral administration of 0.1 mg of methyldigoxin. Five men participated in more than two different studies. Erythrocyte ouabain sensitive efflux rate constant (ERCos) indicated as Na^+, K^+ ATPase activity was measured before and after intravenous or oral administration of digoxin in 5 men for each study.

NE infusion test

During the morning period when the subjects were fasting, indwelling venous catheters were inserted in the antecubital vein and instep vein for blood sampling and *dl*-NE infusion, respectively, through which 5% glucose solutions were slowly infused. Following these procedures, the subjects were placed in a comfortable supine position for 30 min. During this period, blood pressure (BP) was measured by sphygmomanometric

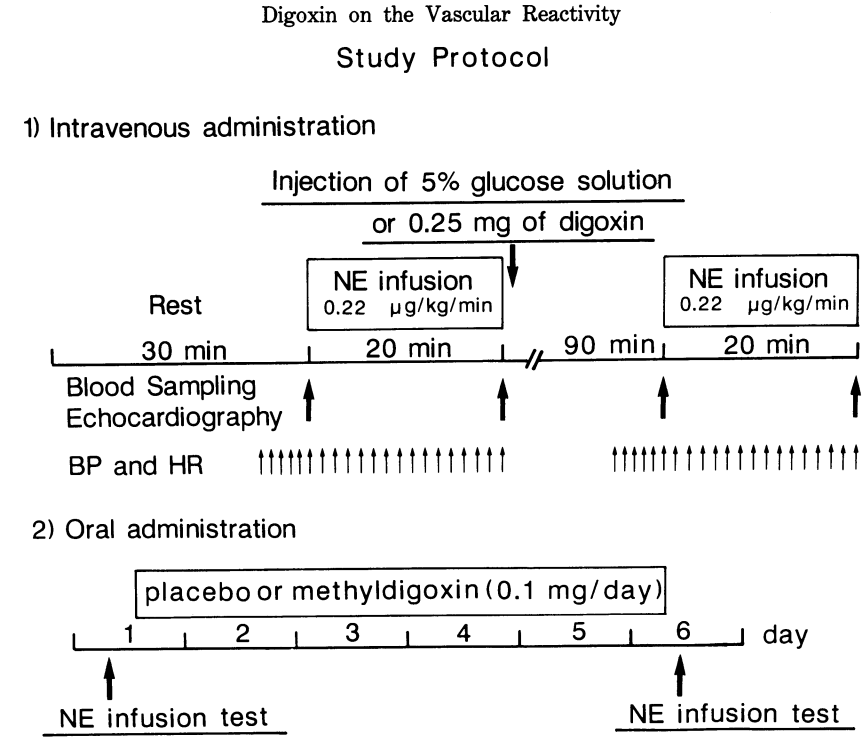

Fig. 1. Study protocol. Abbreviations: NE = norepinephrine; BP = blood pressure; HR = heart rate.

method and heart rate (HR) by electrocardiography at intervals of 1 to 2 min. Echocardiography was then taken for determination of cardiac output (CO) and total peripheral resistance (TPR), and venous blood was drawn for determination of plasma NE concentration (PNE), plasma renin activity (PRA), plasma aldosterone concentration (PAC), intraerythrocytic sodium concentration (ENa), and serum digoxin concentration (SDC). Thereafter, the subjects received an infusion of NE for 20 min at a constant rate of 0.22 μg/kg/min, using an infusion pump TFV1100 (Nihon Koden, Tokyo, Japan). BP and HR were measured every minute during NE infusion, and echocardiography was performed and blood samples for PNE, PRA and PAC were obtained at the end point of NE infusion.

Measurements

Hemodynamic assessment was made by echocardiography (Toshiba SSH-11A with 2.25 MHz transducer, Tokyo, Japan, Honeywell strip chart recorder, Denver, Colorado). CO was calculated by the method of Teichholz[39] and TPR was calculated as mean BP divided by CO.

Immediately after blood sampling, 7 ml of blood for NE assay were transferred to ice-chilled test tubes containing 7 mg of EDTA-2Na and 100 μl of 10% $Na_2S_2O_4$ and plasma was separated by centrifugation at 1,600 *g* for 10 min at 4°C. Three ml of plasma was deproteinized by 3 ml of 0.4 N perchloric acid and adjusted pH 8.4. After extraction of NE from plasma by 20 mg of activated alumina II-III, NE eluated by 100 μl of 0.1 N HCl was detected by an electrochemical method using high performance liquid chromatography L-4000W (Yanaco, Kyoto, Japan)[31].

PRA, PAC and SDC were determined by RIA using a kit with ^{125}I-angiotensin I, ^{3}H-aldosterone and ^{125}I-tyrosyl-digoxin, respectively.

UNaV was calculated from urinary sodium concentration measured by flame photometer (Hitachi 775-A, Tokyo, Japan), with the use of lithium as an internal standard.

ENa was determined by the modified Kaya's method[27]. Three ml of freshly drawn venous blood collected in heparinized tube was centrifuged at 1,600 *g* for 10 min at room temperature. Erythrocytes were then washed twice with cold isotonic $MgCl_2$ solution and injected into a polycarbonate capillary tube (Hematolon, Kayagaki Irikakogyo, Tokyo, Japan). Following centrifugation at 15,000 *g* for 5 min, 30 μl of

packed erythrocytes was transferred into 3 ml of a hypotonic hemolysing solution containing 0.5% LiCl. The concentration of sodium was determined by flame photometer in supernatant of hemolytic solution and expressed in mmol per liter cells.

ERCos was measured by the modification of Cumberbatch and Morgan's method[8]. Nine ml of blood was divided into three tubes. One of them was measured for initial ENa. Ouabain was added to the second tube to a calculated plasma concentration of 10^{-4} mol/liter and an equal volume of distilled water was added to the third tube as control. The latter two tubes were placed in a shaking water bath at 37°C for 2 hr. After incubation ENa was measured. ERCos was calculated by the equation: ERCos (h^{-1}) = Eos / initial ENa /2, where Eos is the difference between ENa after 2 hr incubation in the absence and presence of ouabain.

Evaluation of pressor response and vascular reactivity to NE

The pressor response and systemic vascular reactivity to NE were evaluated using the ratios of increases in mean BP and TPR to increase in PNE during NE infusion, indicated as $\Delta MBP/\Delta PNE$[31,44] and $\Delta TPR/\Delta PNE$, respectively.

Statistical analysis

All the data are expressed as mean ± SD. Statistical analysis was made by nonparametric methods, using the Wilcoxon matched pairs signed rank test. P level less than 0.05 was regarded to be significant.

RESULTS

1) Preliminary study

(1) Preliminary intravenous study

Fig. 2 shows the time course of systolic BP (SBP), diastolic BP (DBP) and HR during NE infusion before and 90 min after injection of 5% glucose solution as placebo in 8 subjects. A constantly infused NE induced a gradual and sustained elevation of SBP and DBP for 20 min, while HR showed a gradual and sustained decrease. Injection of 5% glucose solution did not produce any difference in these parameters. Table 1 summarizes the basal parameters before NE infusion and their changes during NE infusion before and 90 min after injection of 5% glucose solution in the preliminary intravenous study. There was no significant difference in these parameters during NE infusion before and after injection of 5% glucose solution.

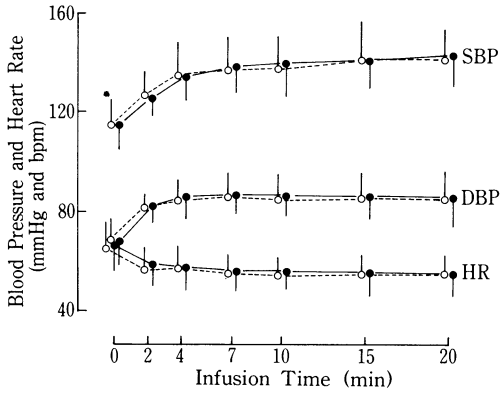

Fig. 2. Time course of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) during NE infusion (0.22 μg/kg/min) before (open circles) and after (closed circles) injection of 5% glucose solution. Each circle and vertical line indicate mean and standard deviation.

(2) Preliminary oral study

In the preliminary oral study as well as the preliminary intravenous study, no significant difference was observed in the basal parameters and their changes during NE infusion before and after 6 days oral administration of placebo (Table 2).

2) Intravenous administration of digoxin

(1) Effects of intravenous administration of digoxin on basal parameters before NE infusion

Table 3 summarizes the basal hemodynamic and chemical parameters before and 90 min after intravenous administration of digoxin in 14 subjects. After intravenous administration of digoxin, SDC was 1.52 ± 0.42 ng/ml and basal hemodynamic parameters remained unchanged except for HR which was significantly decreased from 66.9 ± 8.9 bpm to 63.9 ± 8.2 bpm. Digoxin significantly inhibited ERCos from 0.213 ± 0.020 h^{-1} to 0.207 ± 0.020 h^{-1} and increased ENa from 8.15 ± 0.79 mmol/liter cells to 8.71 ± 0.86 mmol/liter cells. Basal PRA and PAC were unchanged, but PNE was significantly increased from 0.315 ± 0.137 ng/ml to 0.405 ± 0.158 ng/ml.

(2) Effects of intravenous administration of digoxin on the changes in hemodynamic and hor-

Table 1. Basal parameters and their changes during NE infusion before and 90 min after 5% glucose solution as placebo in 8 subjects

	Basal values		Changes during NE infusion	
	Before placebo	After placebo	Before placebo	After placebo
SBP (mmHg)	114.4 ± 9.6	114.6 ± 8.9	25.9 ± 13.5	27.6 ± 12.7
DBP (mmHg)	68.5 ± 8.1	68.0 ± 8.9	16.2 ± 7.8	17.5 ± 6.4
MBP (mmHg)	83.8 ± 7.1	83.5 ± 7.7	19.4 ± 6.7	20.9 ± 6.6
HR (mmHg)	64.5 ± 10.1	66.5 ± 9.8	−9.8 ± 8.7	−9.9 ± 7.5
CO (liter/min)	4.53 ± 0.64	4.56 ± 0.79	−0.49 ± 0.56	−0.49 ± 0.44
TPR (dyne·sec·cm^{-5})	1501 ± 300	1500 ± 320	614 ± 337	604 ± 256
PNE (ng/ml)	0.247 ± 0.082	0.241 ± 0.065	5.033 ± 1.409	5.177 ± 1.131
PRA (ng/ml/hr)	1.15 ± 0.55	1.25 ± 1.14	0.69 ± 0.91	0.60 ± 0.69
PAC (pg/ml)	85.2 ± 25.3	76.6 ± 24.9	17.4 ± 7.3	18.6 ± 8.0
ENa (mmol/liter cells)	8.34 ± 1.05	8.37 ± 1.14	n.d.	n.d.
ΔMBP/ΔPNE (mmHg·ml·ng^{-1})	–	–	4.13 ± 1.75	4.27 ± 1.63
ΔTPR/ΔPNE (dyne·sec·cm^{-2}·ng^{-1})	–	–	124 ± 58	121 ± 52
UNaV (mmol/day)	185.1 ± 26.6			

All values are mean ± standard deviation (SD).

Abbreviations: NE = norepinephrine; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; HR = heart rate; CO = cardiac output; TPR = total peripheral resistance; PNE = plasma NE concentration; PRA = plasma renin activity; PAC = plasma aldosterone concentration; ENa = intraerythrocytic sodium concentration; n.d. = not determined; ΔMBP = increase in MBP during NE infusion; ΔPNE = increase in PNE during NE infusion; ΔTPR = increase in TPR during NE infusion; UNaV = urinary sodium excretion.

Table 2. Basal parameters and their changes during NE infusion before and after 6 days oral administration of placebo in 6 subjects

	Basal values		Changes during NE infusion	
	Before placebo	After placebo	Before placebo	After placebo
SBP (mmHg)	112.7 ± 13.6	113.0 ± 13.7	33.0 ± 9.4	34.8 ± 12.4
DBP (mmHg)	69.6 ± 7.2	72.7 ± 8.7	19.9 ± 7.2	16.9 ± 9.0
MBP (mmHg)	84.0 ± 7.9	86.1 ± 9.2	24.3 ± 6.5	21.9 ± 6.5
HR (bpm)	67.7 ± 10.4	65.3 ± 13.5	−13.7 ± 8.8	−13.9 ± 10.3
CO (liter/min)	4.57 ± 1.00	4.72 ± 0.78	−0.34 ± 0.16	−0.77 ± 0.70
TPR (dyne·sec·cm^{-5})	1515 ± 343	1490 ± 391	761 ± 428	789 ± 475
PNE (ng/ml)	0.305 ± 0.055	0.254 ± 0.089	4.942 ± 2.054	4.609 ± 1.379
PRA (ng/ml/hr)	1.27 ± 1.01	1.35 ± 0.73	0.72 ± 0.42	0.68 ± 0.46
PAC (pg/ml)	100.5 ± 13.5	96.3 ± 17.2	21.8 ± 8.9	18.8 ± 11.5
ENa (mmol/liter cells)	8.93 ± 0.74	8.59 ± 0.93	n.d.	n.d.
UNaV (mmol/day)	178.1 ± 18.9	171.1 ± 25.9	–	–
ΔMBP/ΔPNE (mmHg·ml·ng^{-1})	–	–	5.91 ± 3.71	5.22 ± 2.01
ΔTPR/ΔPNE (dyne·sec·cm^{-2}·ng^{-1})	–	–	180 ± 130	185 ± 113

All values are mean ± SD.

See Table 1 for abbrevations.

monal parameters during NE infusion

Fig. 3 illustrates the effects of intravenous administration of digoxin on increases in systolic BP (ΔSBP), diastolic BP (ΔDBP) and mean BP (ΔMBP) during NE infusion. After intravenous administration of digoxin they were significantly augmented, but a decrease in HR (ΔHR) during NE infusion was not affected (−11.5 ± 4.8 bpm to −12.5 ± 5.5 bpm). Fig. 4 illustrates the effects of intravenous administration of digoxin

Table 3. Basal parameters before and after intravenous administration of digoxin

	before digoxin	after digoxin
SBP (mmHg)	116.8 ± 13.6	114.8 ± 12.1
DBP (mmHg)	70.8 ± 8.7	68.8 ± 8.8
MBP (mmHg)	86.1 ± 9.6	84.2 ± 8.9
HR (bpm)	66.9 ± 8.9	63.9 ± 8.2 *
CO (liter/min)	4.76 ± 0.93	4.66 ± 0.95
TPR (dyne·sec·cm^{-5})	1481 ± 282	1477 ± 276
PNE (ng/ml)	0.315 ± 0.137	0.405 ± 0.158*
PRA (ng/ml/hr)	1.26 ± 0.66	1.29 ± 0.65
PAC (pg/ml)	88.0 ± 18.3	80.0 ± 16.1
ENa (mmol/liter cells)	8.15 ± 0.70	8.71 ± 0.86 **
ERCos (h^{-1})	0.213 ± 0.020	0.207 ± 0.020**
SDC (ng/ml)	–	1.52 ± 0.42
UNaV (mmol/day)	168.8 ± 16.8	

Abbreviations: ERCos = erythrocyte ouabain sensitive efflux rate constant; SDC = serum digoxin concentration. See Table 1 for other abbreviations.
* $p<0.05$, ** $p<0.01$ vs. before digoxin

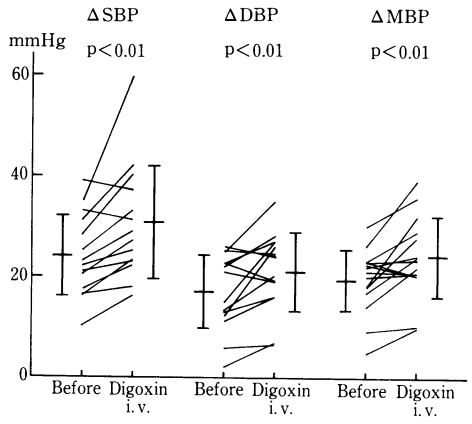

Fig. 3. Effects of intravenous administration of digoxin on the changes in systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP) and mean blood pressure (ΔMBP) during NE infusion.

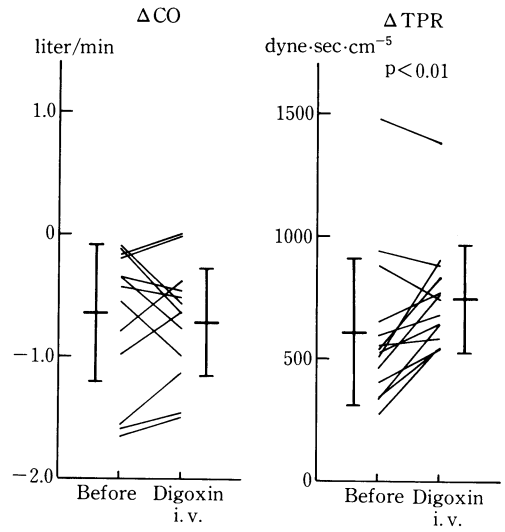

Fig. 4. Effects of intravenous administration of digoxin on the changes in cardiac output (ΔCO) and total peripheral resistance (ΔTPR) during NE infusion.

on changes in CO (ΔCO) and TPR (ΔTPR). ΔCO was unchanged, but ΔTPR was significantly increased from 603 ± 306 dyne·sec·cm^{-5} to 745 ± 342 dyne·sec·cm^{-5}. The increase in PNE (ΔPNE) tended to decrease from 5.498 ± 1.276 ng/ml to 5.324 ± 1.002 ng/ml (Fig. 5). Increases in PRA and PAC (ΔPRA and ΔPAC) during NE infusion were not different before and after digoxin.

(3) Effects of intravenous administration of digoxin on the pressor response and vascular reactivity during NE infusion

Intravenous administration of digoxin significantly augmented the pressor response and vascular reactivity during NE infusion indicated as ΔMBP/ΔPNE and ΔTPR/ΔPNE from 3.81 ± 1.64 mmHg·ml·ng^{-1} to 4.85 ± 2.12 mmHg·ml·ng^{-1} and from 121 ± 75 dyne·sec·cm^{-2}·ng^{-1} to 147 ± 61 dyne·sec·cm^{-2}·ng^{-1}, respectively (Fig. 6).

3) Effects of oral administration of digoxin

(1) Effects of oral administration of digoxin on

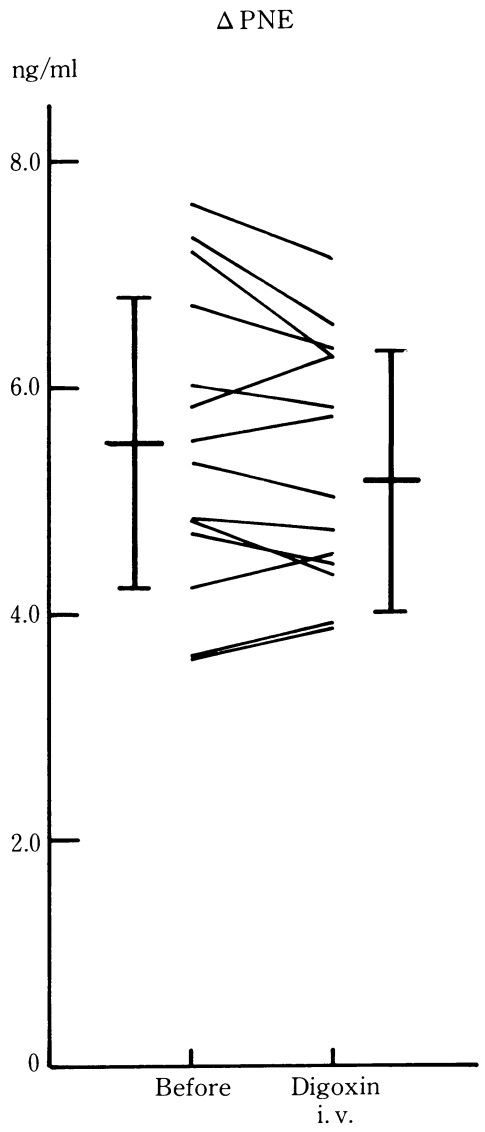

Fig. 5. Effects of intravenous administration of digoxin on the change in plasma NE concentration (ΔPNE) during NE infusion.

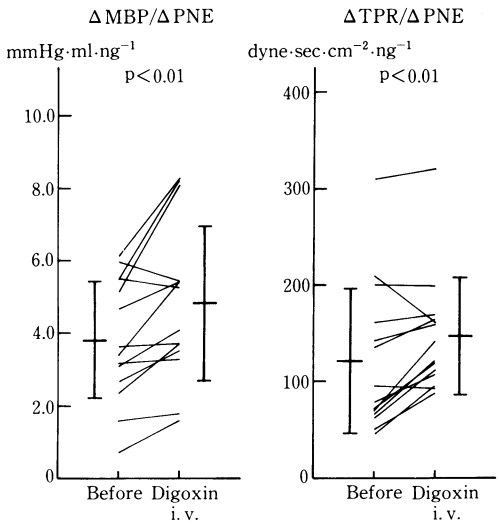

Fig. 6. Effects of intravenous administration of digoxin on the pressor response and vascular reactivity during NE infusion, indicated as ΔMBP/ΔPNE and ΔTPR/ΔPNE, respectively.

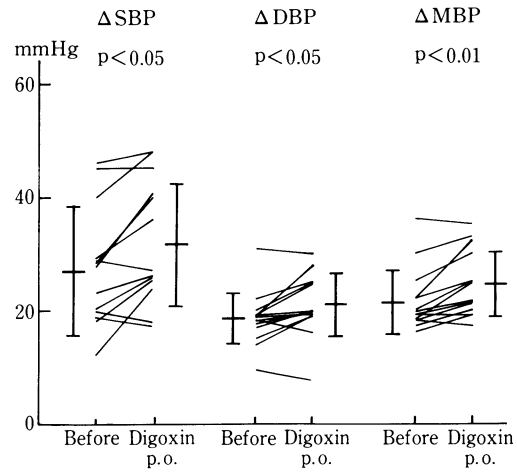

Fig. 7. Effects of oral administration of digoxin on the changes in systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP) and mean blood pressure (ΔMBP) during NE infusion.

basal parameters before NE infusion

As shown in Table 4, after oral as well as intravenous administration of digoxin, SDC was 0.66 ± 0.30 ng/ml and basal HR was decreased from 60.4 ± 6.2 bpm to 55.5 ± 7.0 bpm. ERCos was significantly decreased from 0.213 ± 0.020 h^{-1} to 0.160 ± 0.017 h^{-1} and basal ENa was significantly increased from 8.30 ± 0.71 mmol/liter cells to 9.06 ± 0.99 mmol/liter cells, but the other basal parameters were unchanged.

(2) Effects of oral administration of digoxin on changes in hemodynamic and hormonal parameters during NE infusion

After oral as well as intravenous administration of digoxin, ΔSBP, ΔDBP and ΔMBP were increased (Fig. 7), but ΔHR was unchanged (−13.7 ± 8.8 bpm to −13.9 ± 10.3 bpm). ΔCO

Table 4. Basal parameters before and afrter oral administration of digoxin

	before digoxin	after digoxin
SBP (mmHg)	108.4 ± 6.5	108.6 ± 8.2
DBP (mmHg)	67.9 ± 7.3	66.1 ± 6.9
MBP (mmHg)	81.3 ± 6.2	80.3 ± 6.4
HR (bpm)	60.4 ± 6.2	55.5 ± 7.0 *
CO (liter/min)	4.18 ± 0.64	4.20 ± 0.42
TPR (dyne.sec. cm^{-5})	1579 ± 282	1503 ± 223
PNE (ng/ml)	0.299 ± 0.132	0.317 ± 0.157
PRA (ng/ml/hr)	1.68 ± 0.85	1.43 ± 1.04
PAC (pg/ml)	120.5 ± 52.1	115.4 ± 49.1
ENa (mmol/liter cells)	8.30 ± 0.71	9.06 ± 0.99 **
ERCos (h^{-1})	0.213 ± 0.020	0.160 ± 0.017**
SDC (ng/ml)	–	0.66 ± 0.30
UNaV (mmol/day)	169.5 ± 22.0	176.2 ± 31.9

All values are mean ± SD.
See Table 1 and Table 3 for abbreviations and significances.

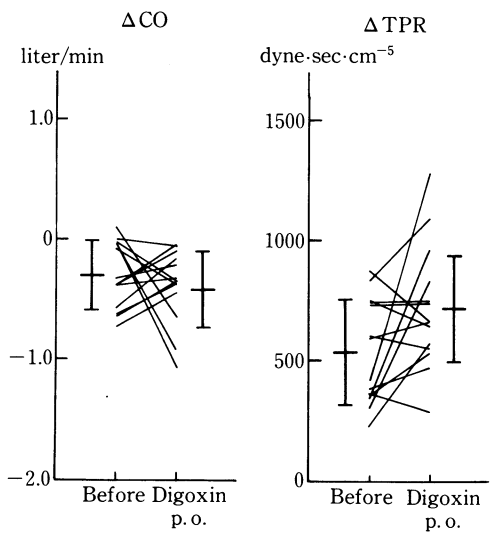

Fig. 8. Effects of oral administration of digoxin on the changes in cardiac output (ΔCO) and total peripheral ressitance (ΔTPR) during NE infusion.

was unchanged, but ΔTPR tended to increase from 533 ± 219 dyne·sec·cm^{-5} to 715 ± 259 dyne·sec·cm^{-5} (Fig. 8). ΔPNE was significantly decreased from 4.420 ± 1.280 ng/ml to 4.148 ± 1.196 ng/ml (Fig. 9). ΔPRA and ΔPAC were unchanged.

(3) Effects of oral administration of digoxin on the pressor response and vascular reactivity during NE infusion

Oral administration of digoxin also significantly augmented the pressor response and vascular reactivity from 5.14 ± 1.70 mmHg·ml·ng^{-1} to 6.25 ± 2.00 mmHg·ml·ng^{-1} and from 130 ± 65 dyne·sec·cm^{-2}·ng^{-1} to 176 ± 53 dyne·sec·cm^{-2}·ng^{-1}, respectively (Fig. 10).

DISCUSSION

In the last decade, abnormalities in sodium transport across cell membrane have been implicated in the pathogenesis of essential hypertension. These abnormalities have been explained by the hypothesis proposed by Dahl[9], Haddy[23], de Wardner[10], Blaustein[4], and many others that essential hypertension is, in part, due to an increase in a circulating sodium transport inhibitor. This hypothesis has been supported by evidence in rats[19,25], dogs[21] and human[11,36]. If this hypothesis is correct, vascular tone may be increased when intracellular sodium concentration is increased by cardiac glycosides. In fact, previous studies have demonstrated extracardiac effects of cardiac glycosides as following findings. The administration of ouabain results in a fall in forearm blood flow and an elevation of forearm vascular resistance and venous tone[30]. Cardiac glycosides enhance the contractile response of excised arterial strips[26] and perfused hindquaters[20] to NE. Vascular smooth muscles have membrane-bound Na^+, K^+ ATPase[15], and inhibition of this enzyme by cardiac glycosides leads to increased intracellular sodium concentration as in myocardium. In the present study, digoxin given either intravenouslly or orally in usual therapeutic doses inhibited ERCos indicated as Na^+, K^+ ATPase activity and increased ENa in normal subjects.

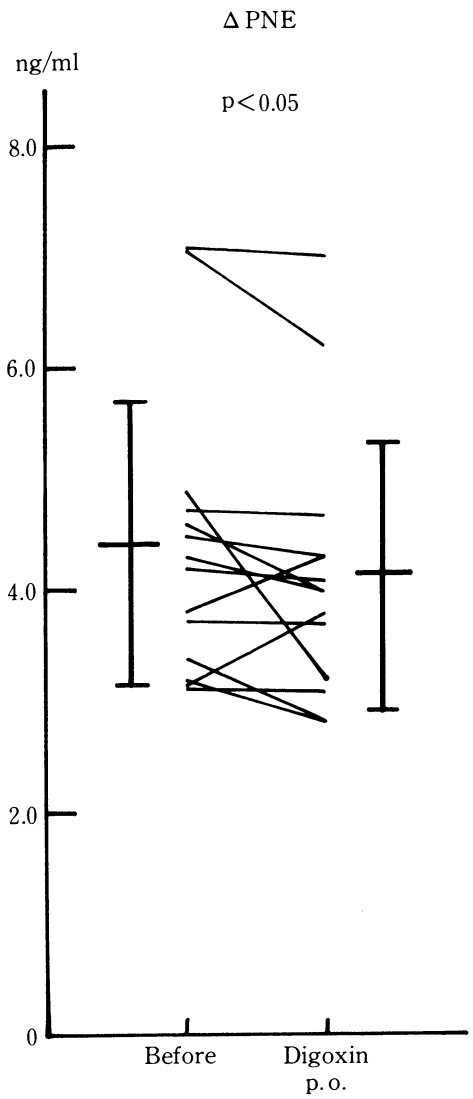

Fig. 9. Effects of oral administration of digoxin on the change in plasma NE concentration (ΔPNE) during NE infusion.

Moreover, digoxin markedly augmented the pressor response and systemic vascular reactivity to NE. Therefore, these findings suggest that augmentation of the pressor response to NE is caused by the enhanced vascular reactivity to NE through inhibition of Na^+, K^+ ATPase activity in cell membrane, this gives support to the above-mentioned hypothesis that a circulating digitalis-like substance is important for blood

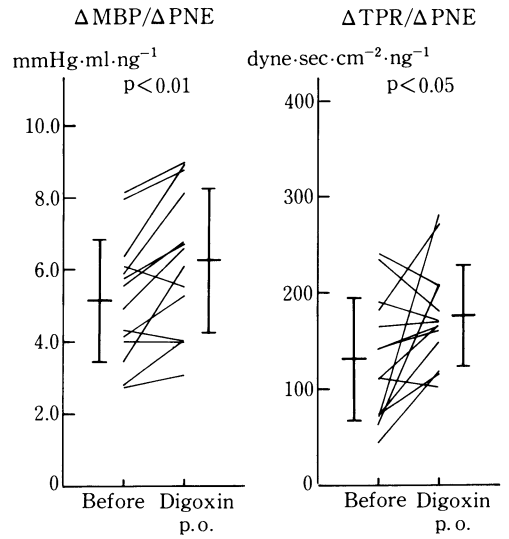

Fig. 10. Effects of oral administration of digoxin on the pressor response and vascular reactivity during NE infusion, indicated as ΔMBP/ΔPNE and ΔTPR/ΔPNE, respectively.

pressure control in humans.

An elevated intracellular sodium concentration leads to an increase in intracellular free calcium concentration[4,38] which is the most important factor regulating vascular smooth muscle tone[7]. Abnormal calcium handling and increased intracellular free calcium concentration have been reported in erythrocytes and platelets of patients with essential hypertension[14]. In our previous study, it was observed that intralymphocytic free calcium concentration showed a positive correlation with intralymphocytic sodium concentration and that both intralymphocytic sodium concentration and free calcium concentration were higher in hypertensive patients than in normotensive controls[34]. In addition, inhibition of calcium inflow using slow channel blocker nifedipine or verapamil blunted the pressor response to NE and angiotensin II in normal subjects[22]. Although intracellular free calcium concentration was not measured in the present study, it is suggested that the augmented vascular reactivity to NE after digoxin may be caused by elevated intracellular free calcium concentration.

Since there is a considerable individual variation in PNE during NE infusion[31,44] and inhibition of Na^+, K^+ ATPase activity by digoxin

affects the function of the adrenergic terminal[5,6,18,32], it is reasonable to use the ratios of increases in MBP and TPR to increase in PNE during NE infusion as the pressor response and systemic vascular reactivity to NE. In the present study, the effects of intravenous administration of digoxin on basal PNE and an increase in PNE during NE infusion were not different from that of oral administration of digoxin. Intravenous administration of digoxin increased basal PNE and tend to decrease an increase in PNE. On the other hand, oral administration of digoxin did not alter the former but decreased the latter. Although digoxin might alter NE kinetics, the pressor response and vascular reactivity to NE were finally augmented.

Either intravenous or oral administration of digoxin augmented the pressor response and vascular reactivity to NE, but basal hemodynamic parameters except for HR remained unchanged in spite of increased basal ENa after digoxin. Furthermore, intravenous digoxin increased basal PNE. The reason for this discrepancy is unclear, but one possible reason is that there is the threshold of a concentration in NE for the augmentation of the pressor response to NE after digoxin. In other words, elevation of blood pressure by digoxin may be necessary for a certain level of sympathetic stimuli.

The possibility that this augmentation of the vascular reactivity might be related to the change in endogenous angiotensin II by digoxin should be considered. In the present study, however, both intravenous and oral administrations of digoxin did not affect the renin-aldosterone axis before and during NE infusion.

Consideration should also given whether the findings observed after digoxin might be related to the effects of repetitive NE infusion or of the placebo. In the preliminary study, however, the basal prameters and their changes during NE infusion were not different before and after administration of placebo.

In summary, either intravenous or oral administration of digoxin increased intraerythrocytic sodium concentration due to inhibition of Na^+, K^+ ATPase activity and augmented the pressor response and vascular reactivity to NE in normal human subjects, but had not effects on basal hemodynamic parameters except for heart rate and the renin-aldosterone axis. These findings indicate that digoxin augments the vascular reactivity to NE through inhibition of Na^+, K^+ ATPase activity in cell membrane, suggesting that a circulating digitalis-like substance may play a key role in the pathogenesis of essential hypertension.

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