PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 401–404 (1980)

Arterial Wall "Waterlogging" Accompanying Chronic Digoxin Treatment in Dogs (40884)[1]

HENRY W. OVERBECK, MOTILAL B. PAMNANI, AND DAVID D. KU

Departments of Medicine, Physiology and Biophysics, and Pharmacology, and the Cardiovascular Research and Training Center, the University of Alabama Medical Center, Birmingham, Alabama 35294, and the Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

Abstract. To chronically decrease activity of the ouabain-sensitive sodium pump in vascular smooth muscle, we administered digoxin (60 μg/kg, followed by 8–16 μg/kg/12 hr, iv) to five male mongrel dogs for 4 weeks. Serum digoxin levels were monitored and maintained at concentrations sufficient to continuously inhibit the sodium pump. Blood pressures and serum Na^+ and K^+ concentrations of the treated dogs did not change significantly. Five paired control dogs received saline injections. Two hours after a final injection, samples of small mesenteric arteries and veins were obtained under sodium pentobarbital (30 mg/kg, iv) anesthesia for measurement of vessel wall ^{86}Rb uptake and water content. In veins there were no significant changes in pump activity or water content. In contrast, in arteries the ouabain-sensitive ^{86}Rb uptake was depressed ($P < 0.02$) in treated dogs. Accompanying this depression was an increase, averaging 6.2% ($P < 0.05$), in mesenteric artery wall water content. These data provide evidence associating chronic digitalis suppression of the sodium pump in arteries with the development of wall edema. These results are compatible with the hypothesis that inhibition of the sarcolemmal sodium pump of arteries may underlie the "waterlogging" of these vessels in hypertension.

Activity of the sarcolemmal sodium pump is reduced in mesenteric vessels of dogs with renal hypertension (1). Accompanying this decreased pump activity in hypertension is an increase in vascular wall content of water, described as "waterlogging" (2). Because the sodium pump plays an important role in maintenance of intracellular volume (3), it has been suggested that these two manifestations of hypertension may be related (1). Thus, in the present study we administered digoxin to dogs for 1 month to produce long-term inhibition of the cell membrane sodium pump. We then measured ouabain-sensitive ^{86}Rb uptake and water content in walls of mesenteric vessels. Myocardial sodium pump activity and [^{3}H]digoxin binding levels were also measured and have been previously reported (4).

Materials and Methods. The methods we used to administer digoxin to dogs and to monitor serum levels and systemic and myocardial effects have been previously described in detail (4). In brief, to five healthy, male, beagle-like dogs weighing 15–20 kg, we gave a priming dose of digoxin (60 μg/kg iv; Lanoxin, Burroughs Wellcome and Co., Research Triangle Park, N.C.), followed by maintenance doses (8–16 μg/kg/12 hr iv) for 4 weeks. The maintenance dose was calculated and adjusted weekly for each dog on the basis of the biological half-life of digoxin in that individual dog, as estimated by measurement of serum digoxin concentrations (Digoxin RIA Kit, Beckman Instrument, Inc., Irvine, Calif.). In three animals (nontoxic group) the maintenance dose of digoxin was adjusted to maintain serum digoxin levels below 4 ng/ml. In two animals (toxic group) higher maintenance levels were used. During the last 4 days of treatment, the maintenance dose of digoxin for the toxic dose group was reduced so that serum digoxin concentrations were comparable in both groups at the time of sacrifice. Five control

[1] This study was supported by U.S. Public Health Service Research Grants HL-23600, HL-23312, and HL-16052. Portions of this work have been published in abstract form (Proc. Int. Union Physiol. Sci. 13, 573, 1977).

0037-9727/80/080401-04$01.00/0

dogs received saline injections and were pair fed with the dogs receiving digoxin. Serum sodium and potassium concentrations at time of sacrifice were measured by flame photometer, arterial pressure was measured weekly by femoral arterial puncture, and Lead II EKG was recorded on alternate days.

After 4 weeks of treatment, cardiovascular tissue was obtained from digoxin-treated and paired control dogs under pentobarbital anesthesia (30 mg/kg iv). The dogs were sacrificed 2 hr after receiving a final injection of 60 μg/kg digoxin iv (or saline in control dogs). We harvested most small (0.3–1.0 mm o.d.) arteries and 0.5 to 6.0 mm o.d. veins from the jejunum and ileum using methods similar to those we have described (1). In each dog we started at the ileocecal junction and worked proximally to the duodenum, carefully removing each vascular arcade, in turn, up to the border of the intestine. These vessels were gently dissected free from adventitia, split longitudinally, blotted once with cotton gauze to remove blood, and placed in Krebs–Henseleit solution at room temperature. Within 30 min, the specimens were placed in a 0°C potassium-free Krebs–Henseleit solution ($NaHCO_3$, 27.2 m*M*; NaCl, 117.0 m*M*; $NaHPO_4 \cdot H_2O$, 1.0 m*M*; $MgSO_4 \cdot 7H_2O$, 1.2 m*M*; $CaCl_2$, 2.5 m*M*; and glucose, 11.1 m*M*), aerated with 95% O_2–5% CO_2, and incubated for 2 min to further load the tissue with sodium. Next, the artery or vein tissue was incubated in aerated potassium-free Krebs–Henseleit solution at 37°C for 2 min. This solution contained "cold" RbCl, 2 m*M*. For this incubation the pooled arterial or venous tissues were randomly divided in half. One-half was incubated in the medium without ouabain and the other half in the medium with added ouabain (0.2 m*M*). ^{86}RbCl (specific activity 1.3–4.7 mCi/mg, New England Nuclear) was added to each medium to a standard concentration (0.01–0.03 m*M*, depending on specific activity level) and the incubation was continued for another 16 min. Tissue was then washed three times (total time 30 sec) with Krebs–Henseleit containing 2 m*M* "cold" RbCl, blotted with tissue paper to remove surface fluid, weighed, and placed in a crystal scintillation counter to determine ^{86}Rb uptake (pmol/mg tissue wet wt). "Specific uptake" (ouabain-sensitive uptake) was calculated as the difference between the ^{86}Rb uptake without ("total" uptake) and with ("nonspecific" uptake) ouabain. Student's *t* test was used to compare group means of uptakes (5). *P* values ≤ 0.05 were considered significant.

Other similar vessels were simultaneously obtained for measurement of water content. Using techniques similar to those we have previously described (6), we rapidly excised these vessels. They were then placed on a glass plate, cleaned, and opened longitudinally. The tissues were blotted once with filter paper to remove blood and surface fluid, and quickly weighed to the nearest 0.01 mg. All these procedures were done at room temperature. The tissue was then oven-dried at 100°C for 24 hr, and, when again cooled to room temperature, reweighed. The difference between these wet and dry weights was considered water content and was expressed as milliliters per kilogram of wet weight. One-tailed Student's *t* test was used to compare group means of water contents. Again, *P* values ≤ 0.05 were considered significant.

Results. The biological half-life of digoxin in these dogs, estimated from serum digoxin concentrations at 6, 12, and 24 hr after injection, was 21.8 ± 2.9 hr ($N = 7$). Three dogs received 8–12 μg digoxin/kg/12 hr and had measured serum digoxin concentrations ranging from 0.5 to 4 μg/ml over the 4-week period. These dogs, and their pair-fed controls remained healthy. In two treated animals receiving higher dose levels of digoxin (14–16 μg/kg/12 hr) measured serum digoxin concentrations ranged from 1 to 10 μg/ml over the 4-week period. In these two dogs there were intermittent toxic effects with vomiting, reduced food intake, weight loss, and first and second degree heart block. When these toxic effects were noted, the dose of digoxin was immediately reduced to prevent unnecessary discomfort. A veterinarian from Labo-

ratory Animal Services participated closely in the care of these dogs.

Femoral arterial pressures in the five treated dogs (115.0 ± 3.8 mmHg; mean ± SEM) were not significantly different from those in control dogs (125.0 ± 4.5). At time of sacrifice, serum sodium and potassium concentrations in the experimental and control dogs were also not significantly different.

Figures 1 and 2 present total, ouabain-insensitive (nonspecific), and ouabain-sensitive (specific) uptakes by mesenteric arteries and veins. In mesenteric arteries, the total and specific uptakes were significantly reduced in dogs receiving digoxin. This was true whether the paired (as in the figures) or nonpaired ($P < 0.02$) t test was used to compare groups. In contrast to arteries, there were no statistically significant differences in uptakes in mesenteric veins. In neither arteries nor veins was there evidence for differences in the nonspecific uptake, representing passive ^{86}Rb influx, nonspecific binding, and ^{86}Rb in interstitial fluid.

Figures 3 and 4 present wall water contents in these vessels. Digoxin treatment was accompanied by increases, averaging 6.2% in wall water content of mesenteric arteries ($P < 0.02$ by paired and $P < 0.05$ by unpaired t test). In contrast, no significant differences were observed in venous wall water contents ($P > 0.5$).

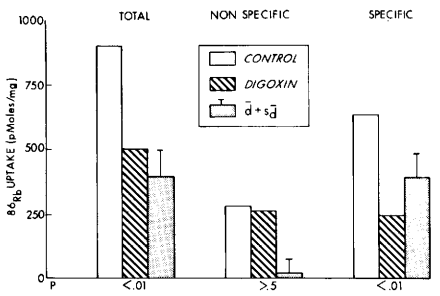

FIG. 1. ^{86}Rb uptake by ileal and jejunal arteries of digoxin-treated (cross-hatched bars) and control (open bars) dogs. Stippled bars represent mean differences + SEM for total, nonspecific (ouabain-insensitive), and specific (ouabain-sensitive) uptakes. P values provided for comparison of values in paired digoxin-treated and control dogs.

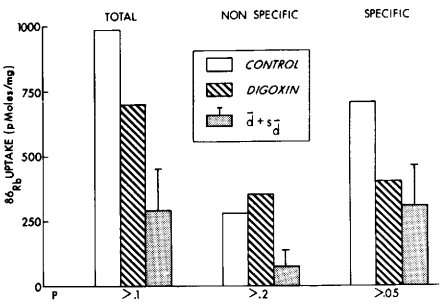

FIG. 2. ^{86}Rb uptake by ileal and jejunal veins. As in Fig. 1.

Discussion. As previously reported (4), the degree of inhibition of the myocardial sodium pump in these dogs correlated with serum digoxin levels and with degree of binding of digoxin to myocardial Na^+, K^+-ATPase molecules. After 4 weeks of digoxin treatment, these relationships remained unchanged, indicating that prolonged digoxin treatment was accompanied by continuing inhibition of enzyme and pump. Similarly, in arterial tissue from these same dogs, reported here, we have provided evidence that, after 4 weeks of treatment, digoxin continues to inhibit the pump.

Such pump suppression in arteries would be expected to increase the contractile state of the vascular smooth muscle, thereby elevating arterial resistance (1, 7). But, in

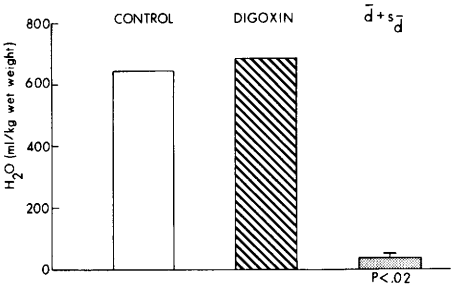

FIG. 3. Water content of walls of ileal and jejunal arteries of digoxin-treated (cross-hatched bar) and control (open bar) dogs. Stippled bar represents mean difference + SEM. P value provided for comparison of values in paired digoxin-treated and control dogs.

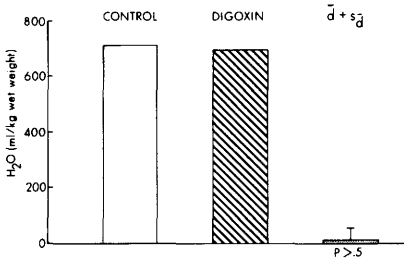

FIG. 4. Water content of walls of ileal and jejunal veins. As in Fig. 3.

these dogs there was no evidence for changes in arterial blood pressure accompanying the digoxin treatment. One must consider, however, that changes in body fluid volumes accompanied digoxin treatment in these dogs, especially in the dogs becoming toxic. Thus, a reduced cardiac output may have prevented the development of hypertension, even though the peripheral resistance was probably elevated (8, 9).

Associated with pump inhibition in the mesenteric arteries of these dogs were significant increases, averaging 6.2%, in the wall water content. This vascular wall "waterlogging" is quantitatively similar to that occurring in most forms of hypertension (2). In hypertension at least a portion of this increased water is reported to be intracellular in location (10). It has been suggested that such "waterlogging" is the effect of increased intravascular pressure in hypertension (11), but, elevated arterial pressure was not responsible for the increases in wall water content we observed in the present investigation.

The role of the membrane sodium pump in regulation of cellular volume in vascular tissue was previously studied by Daniel and Wolowyk (12). In acute experiments *in vitro*, these investigators found that ouabain or a K^+-free medium was unable to change water content of rabbit aortic strips. In the presence of the metabolic inhibitors iodoacetate and dinitrophenol, in contrast, the vascular tissue gained water. On the basis of these acute experiments the investigators concluded that the ouabain-sensitive sodium pump probably does not play an important role in volume regulation in vascular tissue. It appears from the present experiments, however, that sarcolemmal pump inhibition, if chronic rather than acute, does significantly affect volume regulation in vessel walls in dogs. Because such chronic pump inhibition apparently occurs in hypertension (1, 7), the results of the present study would support the hypothesis (1) that inhibition of the sarcolemmal sodium pump may be involved in the mechanism of the vascular wall "waterlogging" that accompanies hypertension.

1. Overbeck, H. W., Pamnani, M. B., Akera, T., Brody, T. M., and Haddy, F. J., Circ. Res. **38** (Suppl. II), 48 (1976).
2. Tobian, L., Physiol. Rev. **40**, 280 (1960).
3. MacKnight, A. D. C., and Leaf, A., Physiol. Rev. **57**, 510 (1977).
4. Ku, D. D., Akera, T., Brody, T. M., and Weaver, L. C., Naunyn–Schmiedeberg's Arch. Pharmacol. **301**, 39 (1977).
5. Steele, R. G. D., and Torrie, J. H., Principles and Procedures of Statistics. New York, McGraw–Hill, 1960.
6. Pamnani, M. B., and Overbeck, H. W., Circ. Res. **38**, 375 (1976).
7. Overbeck, H. W., Amer. J. Physiol. **223**, 1358 (1972).
8. Mason, D. T., and Braunwald, E., J. Clin. Invest. **43**, 532 (1964).
9. Vatner, S. F., Higgins, C. B., Franklin, D., and Braunwald, E., Circ. Res. **28**, 470 (1971).
10. Villamil, M. F., Medicina **32** (Suppl. I), 57 (1972).
11. Hollander, W., Kramsch, D. M., Farmelant, M., and Madoff, I. M., J. Clin. Invest. **47**, 1221 (1968).
12. Daniel, E. E., and Wolowyk, M. W., J. Physiol. **214**, 20P (1971).

Received November 9, 1979. P.S.E.B.M. 1980, Vol. 164.