Bovine Adrenals Contain, in Addition to Ouabain, a Second Inhibitor of the Sodium Pump*

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In the search for endogenous cardiac glycosides, two different inhibitors of the sodium pump have been isolated from bovine adrenals. Inhibitor A with a molecular mass of 600 Da and a UV maximum at 250 nm was purified from 16 kg of bovine adrenals. The pure substance (<1 ng) inhibited the sodium pump of human red blood cells with an affinity similar to that of ouabain, yet it cross-reacted with antibodies against the bufadienolide proscillaridin A but not against the cardenolide ouabain. Inhibitor A was slightly more hydrophilic than ouabain on RP-C18 high pressure liquid chromatography. Hence, it showed properties similar to the proscillaridin A immunoreactivity (Sich, B., Kirch, U., Tepel, M., Zideck, W., and Schoner, W. (1996) Hypertension 27, 1073–1078) that increased in humans with systolic blood pressure and pulse pressure.

Inhibitor B of the sodium pump with a molecular mass of 584 Da was purified 10⁴-fold from 20 kg of bovine adrenals. It cross-reacted with antibodies against ouabain but not with antibodies against proscillaridin A and inhibited the sodium pump of human and rat red blood cells with the same affinity as ouabain. All other properties, such as the retention time in a C₁₈-reversed phase chromatography, molecular mass determination by electrospray mass spectrometry and fragmentation pattern, and UV and ¹H NMR spectroscopic data, were identical to ouabain. Hence, sodium pump inhibitor B from bovine adrenals is the cardenolide ouabain.

Since the cardiac glycoside binding site of the sodium pump has been conserved over the millennia, it has been suggested that endogenous cardiac glycosides may exist. In fact, a material cross-reacting with antibodies against ouabain, digoxin, and digitoxin and inhibiting the sodium pump circulates in increased concentrations in the blood of patients with low renin essential hypertension (1–4). There has been an intensive search for such substances that has led to the isolation of an ouabain-like inhibitor of the sodium pump from human plasma (5) and an attempt to isolate an isomer of ouabain from bovine hypothalamus (6). Nanogram scale CD structural analysis indicates that both substances show similar properties but are not identical with ouabain (7). It has been shown in a number of investigations that both compounds show inotropic effects and increase the contractility of arterial blood vessels (8, 9). They may, therefore, be a new type of steroid hormone involved in the genesis of low renin essential hypertension (2, 3, 8).

Consistent with such a possibility, the release of material cross-reacting with ouabain antibodies has been demonstrated in vitro from zona glomerulosa cells of bovine adrenals upon the addition of ACTH and angiotensin II (10, 11). Additionally, other cardenolides have been isolated from human and rat urine, namely a substance indistinguishable from digoxin from human urine (12) and neoconvalloside as well as the monoharmnosaide of periplogenin from rat urine. The latter two compounds cross-reacted with anti-ouabain and anti-digoxin antibodies but originated in the diet of the rats (13). A substance of unknown nature and indistinguishable in its mode of action from ouabain, but differing in its molecular mass (below 350 Da), was purified from bovine adrenals (14). This substance, called adrexin C, differed in its properties from another yet unidentified inhibitor of the sodium pump, called uroxin, that was isolated from pig urine and had a molecular mass of 620 Da (15). Therefore, it was concluded that at least two different types of endogenous inhibitors of the sodium pump in the mammalian body exist (16). In fact, other inhibitors of the sodium pump of the bufadienolide type of cardiotonic steroids have been identified; a substance was isolated from human cataractous lenses that was identified as 19-norbufalin and a peptide derivative thereof (17). A compound similar to the amphibian bufadienolide marinobufagenin was isolated and identified from human urine (18), and a substance from human placentas with a molecular mass of 370 Da was tentatively identified as a dihydropryne-substituted steroid, possibly a 3β,14α,20:21-bufadienolide (19).

Starting from the idea that mammals may, like toads, be able to synthesize bufadienolides, we recently demonstrated that a compound is circulating in increased concentrations in patients with hypertension, which cross-reacts with antibodies against proscillaridin A (20). The compound differed in its mode of action from ouabain insofar as its concentration did not correlate with the diastolic blood pressure, albeit it had a similar HPLC retention time to that of ouabain. It was clearly neither ouabain nor marinobufagenin, since the antibodies against proscillaridin A did not cross-react with these cardiotonic steroids. Since bovine adrenals have been identified as a gland where synthesis and release of cardiotonic steroids may occur (5, 9, 10), we intended to isolate this substance and to

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* The abbreviations used are: HPLC, high pressure liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry; ACTH, adrenocorticotropic hormone.

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show that the material cross-reacting with anti-proscillaridin A-IgG differs from the known endogenous inhibitors of the sodium pump. The work presented here shows that besides ouabain an additional inhibitor of the sodium pump of molecular mass 600 Da can be isolated. This compound cross-reacts with antibodies against proscillaridin A but from its different UV spectrum does not seem to be a bufadienolide or a cardenolide. A preliminary report has been given in abstract form (21).

MATERIALS AND METHODS

Antibodies against ouabain from rabbits were a kind gift of Prof. R. E. Lang (Dept. of Physiology, University of Marburg, Germany). Immunochemicals were obtained from Jackson Immunoresearch and Dianova (Hamburg), â9RbCl was from Amersham-Buchler (Braunschweig), and silicon oil AR 200 and 20 were from Wacker Chemie (München). All other chemicals were of the highest available purity.

ASSAYS

Inhibition of â9RbCl Uptake into Red Blood Cells—This was essentially performed as described previously using a silicon oil centrifugation technique in Eppendorf cups (20, 22).

Determination of the Cross-reactivity with Antibodies against Proscillaridin A and Ouabain—This was performed essentially as described by Sich et al. (20). The properties of the antibodies against proscillaridin A and ouabain from rabbits are shown in Table I.

Mass Spectrometry and 1H NMR Spectroscopy—Molecular masses were determined by electrospray mass spectrometry on TSQ 700 mass spectrometers (Finigan, San Jose, CA) at the Deutsches Krebsforschungszentrum in Heidelberg and the Gesellschaft für Biotechnologische Forschung in Braunschweig, respectively. 1H NMR spectra were recorded at 300 K on a Bruker DMX-600 NMR spectrometer locked to the major deuterium resonance of the solvent, CD3OD.

Purification of the Inhibitors of the Sodium Pump from Bovine Adrenals

Purification of Sodium Pump Inhibitor A—Bovine adrenals (16 kg) were collected at the Giessener Schlachthof, freed from surrounding fat, and stored at −18 °C until use.

Methanol Extraction—Portions of 2 kg of the frozen tissue were homogenized for 3 min in 2.5 liters of methanol in a blender. The mixture was centrifuged at 4 °C for 20 min and 9,000 × g in a rotor GS3 of a Servall RC2B refrigerated centrifuge. The supernatant was decanted. The sediment was resuspended in the same volume of methanol and stirred for 60 min at room temperature. The mixture was centrifuged a second time, and the combined supernatants were brought to dryness using a Buchi rotary evaporator. This extraction of 16 kg of bovine adrenals resulted in 297 g of a dark brown greasy material.

Acetone Extraction—This residue was suspended in acetone and stirred for about 20 min. After centrifugation for 10 min at 6,500 × g, the supernatant was decanted and concentrated by rotary evaporation. The sediment was extracted again with acetone in the same way, and this procedure was repeated until the yellow color had disappeared.

Ultrafiltration of the Sediment from the Acetone Extraction—The sediment fraction (84.5 g) was suspended in 5 liters of distilled water, and the suspension was filtered through an OMEGA polysulfon membrane filters with an exclusion size of 10, 3, and 1 kDa using a Filtron Pro Vario-3-System and a pressure <3 bar. The filtrate that had passed the filter with a 1-kDa exclusion volume was lyophilized.

Flash Chromatography—The residue was dissolved in ∼100 ml of 70% methanol. Any insoluble material was removed by Schleicher & Schuell 355F filters. The fluid was added to a 5 × 15-cm Bakersil™ Octadecyl-C18-column (40-µm particle size) that had been equilibrated with water. After washing with 4,000 ml of distilled water at a flow rate of 25 ml/min, the inhibitor was eluted with 2,000 ml of 70% methanol. Fractions eluting at 2,000 ml of 80, 90, and 100% methanol showed no inhibitory potency. The 70% methanol fraction was brought to dryness by rotary evaporation.

First HPLC Chromatography on Lichrospher 100-5-CN—The residue dissolved in a small amount of distilled water was applied in small volumes to a 20 × 250-mm column filled with Lichrospher 100-18-10-RP-CN (10-µm particle size). A linear 0–100% methanol gradient in 60 min was applied at a flow rate of 4 ml/min. A Merck-Hitachi HPLC with L-6200 Intelligent Pump, L-3000 photodiode array detector, LC-Orga- nizer, and D-2000 Chromato-Integrator was used. The absorbance at 220 nm was recorded, and 4-ml fractions were taken. The inhibitor eluted at 44–49-min retention time.

Second HPLC Chromatography on Lichrospher 100-5-CN—The concentrated fractions of the inhibitor were rechromatographed on the same column using a linear 5–30% acetonitrile gradient containing 0.1% trifluoroacetic acid in 70 min and a flow rate of 2 ml/min. Fractions (2 ml) were collected, and samples eluting with a retention time of 45–46 min were concentrated by evaporation.

Third and Fourth HPLC Chromatography on a Nucleosil-Phenyl Column—The residue was dissolved in a small amount of distilled water and applied on to a Nucleosil-120-7-C8 column (7-µm particle size, 4 × 250 mm), and a shallow gradient of acetonitrile in 0.1% trifluoroacetic acid was applied at a flow rate of 1 ml/min. A linear gradient of 5–10% acetonitrile in 10 min, isocratic elution at 10% acetonitrile for 40 min and then increased to 30% acetonitrile within the next 10 min and thereafter up to 100% acetonitrile within the next 10 min). The inhibitor eluted with a retention time of 13–14 min. This step was repeated using the fraction with a retention time of 13–14 min. The fractions eluting at 13–14-min retention time were concentrated.

Fifth HPLC Chromatography on Lichrospher 100-5-CN—Although the fraction obtained in the previous step seemed homogeneous when recorded at 220 nm, ESI-MS analysis revealed major impurities. Therefore, the material was applied onto a 4 × 250-mm column of Lichrospher 100–5–NH2 (5-µm particle size), and a descending gradient of acetonitrile in 0.1% trifluoroacetic acid (100–90% within 10 min, flow rate, 1 ml/min; recording wave length, 250 nm) was used. The substance eluted heterogeneously in the void volume. Fractions with a retention time of 2–3 min were concentrated.

Sixth HPLC Chromatography on Lichrocart 100-3-CN—The residue was taken up in distilled water and applied onto a 4 × 250-mm Lichrocart 100-3-CN (3-µm particle size), and an isocratic elution with 1% ammonium acetate, pH 5.8 (89 parts), acetonitrile (10 parts), and dioxane (10 parts) was started with a flow rate of 0.5 ml/min. Fractions were recorded at 220 and 250 nm. The inhibitor eluted at 13.5 min and an impurity at 11.5 min.

Seventh HPLC Chromatography on Nucleosil 100-3 RP 18—The fraction eluting at 13.5-min retention time was applied onto a 4 × 250-mm Nucleosil 100-3 RP 18 column (3-µm particle size) and eluted with 20% acetonitrile containing 0.1% heptafluorobutyric acid at a flow rate of 0.5 ml/min. The eluate was recorded at 220 and 250 nm, and the fraction with a retention time of 6.6 min was concentrated.

Purification of Sodium Pump Inhibitor B

Methanol Extraction—Frozen bovine adrenals (20 kg) were homogenized in 1-kg portions with 5 liters of methanol in a blender for 3 min. The homogenate was centrifuged for 20 min at 4 °C. The supernatant was decanted, and the sediment was extracted again with 5 liters of methanol with stirring for 60 min. After sedimentation of the insoluble material under the above conditions, supernatants were combined and concentrated by rotary evaporation. A brownish gluey material (615 g) was obtained.

Soxhlet Extraction with Chloroform—The residue from the above

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**Table I**

| Substances          | Anti-ouabain IgG | Anti-proscillaridin A IgG |
|---------------------|------------------|--------------------------|
| Cardenolides        |                  |                          |
| Ouabain             | 100              | 0                        |
| K-strophanthidin    | 16.67            | 0.083                    |
| Digtotoxigenin      | 9.35             | ND                       |
| Digtotoxin          | 0                | 0.46                     |
| Digoxin             | 0.19             | 0                        |
| Convallatoxin       | 1.8              | 0.054                    |
| Bufadienolides      |                  |                          |
| Proscillaridin A    | 7.75             | 100.0                    |
| Bufalin             | ND               | 84.4                     |
| Marinobufagenin     | ND               | 5.0                      |
| Other steroid hormones |               |                          |
| Cortisol            | ND               | 0                        |
| Estradiol           | 0                | 0                        |
| Testosterone        | ND               | 0.1                      |
| Progestosterone     | ND               | 0.125                    |
| Corticosterone      | ND               | 0                        |

1 ND, not determined.
was subjected in fractions of 12.5 g to a Soxhlet extraction with 1 liter of chloroform. The procedure was terminated when the chloroform phase in the extracting funnel was no longer brownish in color. The defatted residue (250 g) was then removed from the paper funnel and dried. This residue was extracted with ethanol and gave 45 g after flash evaporation.

Ultrafiltration—This material was taken up in ethanol, diluted with water, and subjected to ultrafiltration under the conditions described for compound A. The filtrate with a molecular size <1,000 Da gave 44 g of a powdery dark brown material upon lyophilization.
First HPLC Chromatography on Lichrospher 100-RP 18—The residue was taken up in distilled water and applied to a 20 × 250-ml column filled with Lichrospher 100-18-10-RP-C18 (particle size: 10 μm) of a GAT high pressure liquid chromatograph equipped with LC 1110 HPLC pump, DI 510 interphase, LC 11210 UV-visible detector, LC 1431 organizer, LCD 501 UV-visible detector, and a DP900 chromatography data station. The column was run at 4 ml/min for 60 min with a 0–100% methanol linear gradient containing 0.1% trifluoroacetic acid, and the eluate was recorded at 220 nm. Fractions of 4 ml were collected, and the inhibition of $^{86}$Rb$^+$ uptake into human red blood cells was measured for the individual fractions after the evaporation of methanol. Fractions with a retention time of 70–100 min were combined and dried (yield: 5.9 g).

Second HPLC Chromatography on Lichrospher 100-RP 18—The fractions with inhibitory action (retention time: 44–49 min from the previous purification step) were dissolved in distilled water and applied again onto the same column. A methanol gradient with a flow rate of 10 ml/min was applied, which increased within 15 min from 5 to 30% methanol, followed by a steep rise to 95% methanol to 40-min retention time. Fractions (10 ml) were collected. The inhibitor eluted at a retention time of 3–37 min and was dried (yield: 3.2 g).

Third HPLC Chromatography on Lichrospher 100-RP 18—The inhibitor obtained from the previous step was dissolved in distilled water and applied again onto the same column. A linear methanol gradient with a flow rate of 10 ml/min was applied at an initial concentration of 5% methanol, increased to 65% methanol within 25 min, and increased subsequently to 95% methanol within the next 5 min. The inhibitor eluted with a retention time of 21–22 min. The fractions were combined and lyophilized (yield: 127 mg).

Fourth HPLC Chromatography on Lichrospher 100-RP 18—To refine the purification, the inhibitor was dissolved in distilled water and...
applied onto the same preparatory column, and a shallow linear gradient from 5 to 35% methanol within 60 min was applied. The inhibitor eluting with a retention time of 58–63 min was evaporated (yield: 6 mg).

Fifth HPLC Chromatography on Nucleosil 120-7-C6H5—The inhibitor was dissolved in distilled water and applied onto a 4 × 250-mm column filled with Nucleosil 120-7-C6H5 (particle size: 7 μm). The
column was eluted at a flow rate of 1 ml/min with 5% methanol for 15 min, followed by a linear rise for 10 min to 10% methanol and then by a linear rise to 30% methanol within the next 30 min. Fractions of 1 ml were collected, and the inhibitor was found at 33.7–36.0-min retention time (yield: 1.19 mg).

**Sixth HPLC Chromatography on a Hypersil Phenyl Column**—To achieve better resolution, the sample was rechromatographed on the same type of column (4 x 150 mm) with a smaller particle size (5 μm) using the same gradient. Two peaks were detected with a retention time of 47.2 and 52.3 min. These were collected and evaporated (yield: 71 μg).

**Seventh HPLC Chromatography on a Nucleosil 100-3 RP 18 Column**—The fraction with a retention time of 52.3 min from the previous chromatography was dissolved in 100 μl of H2O, and aliquots of 20 μl were chromatographed on a 4 x 250-mm column filled with Nucleosil 100-3 RP 18 (3-μm particle size), and a gradient with a flow rate of 0.3 ml/min of 5–95% methanol in 60 min was applied. A single peak with a retention time of 47.2 min was visible at 220 nm, which was again collected by hand and evaporated (yield: 20 μg).

**RESULTS**

**Purification of the Sodium Pump Inhibitor A**—This type of inhibitor of the sodium pump could be purified after extraction from 16 kg of bovine adrenals in 11 successive steps including seven HPLC chromatographic stages. Fig. 1 gives an overview of the purification efficiency of the individual steps. The compound was absorbed by octadecyl and phenyl residues attached to the HPLC column matrix (HPLC purification steps 1–4 of Fig. 1) but eluted in the void volume of a LiChrospher 100-5-NH2 column and was therefore not anionic under the conditions applied. However, the inhibitor binds to CN-column material that has some specificity for compounds with double bonds (23) (sixth HPLC chromatography, Fig. 1). The compound eluting with a retention time of 13.5 min was apparently already pure, since it was not possible to separate any additional compounds in a reversed phase chromatography on Nucleosil 100-3-RP 18 (Fig. 1, last chromatography). It is evident from a comparison of the inhibitor’s chromatographic behavior with that of ouabain that the sodium pump inhibitor A is somewhat more hydrophilic than ouabain (Fig. 1, last chromatography). The yield of the last purification step was so low, however, that weighing was not possible.

**Properties of the Sodium Pump Inhibitor A from Bovine Adrenals**—The purified inhibitor inhibited half-maximally 86Rb1 uptake into human red blood cells with an affinity similar to that of ouabain (I50 = 5.3 ± 10-8 M) (Fig. 2). Unfortunately, the isolated substance could not be weighed. Therefore, this conclusion is based on the assumption that the molar absorbance of inhibitor A is similar to that of ouabain (I50 = 5.4 ± 10-8 M) (Fig. 3).

**Fig. 7.** Comparison of the concentration dependence of inhibitor B from bovine adrenals with that of ouabain in the inhibition of the sodium pump of human and rat erythrocytes. The 86Rb1 uptake within 60 min in the absence of steroids or inhibitor was set to 100% at 37 °C. Each point represents the mean value of three experiments. A, effect of inhibitor B on 86Rb1 uptake into human red blood cells. Half-maximal inhibition was obtained at 8.8 ± 10-8 M of inhibitor B (ouabain: 8.2 ± 10-8 M). B, effect of inhibitor B on 86Rb1 uptake into rat red blood cells.

**Fig. 8.** Comparison of the interaction of the inhibitor B from bovine adrenals with antibodies against ouabain and proscillaridin A. Interaction with proscillaridin A antibodies (left) and ouabain antibodies (right) in an indirect competitive enzyme-linked immunosorbent assay is shown. Left, Proscillaridin A, I50 = 4.7 ± 10-8 M; Inhibitor B, I50 = 1.3 ± 10-8 M (0.3% cross-reactivity). Right, Ouabain, I50 = 7.4 ± 10-8 M; Inhibitor B, I50 = 7.9 ± 10-8 M (94% cross-reactivity);
That inhibitor A is different from ouabain was also evident from its interaction with antibodies against ouabain and proscillaridin A (Fig. 4). The inhibitor binds to antibodies against proscillaridin A but not against ouabain. Consistent with this, we were able in preliminary experiments to purify inhibitor A partially by affinity chromatography on a column matrix containing bound anti-proscillaridin A IgG (not shown). Also, negative ion electrospray mass spectroscopy indicated that sodium pump inhibitor A is different from ouabain, since it has a molecular mass of 600 Da determined from the deprotonated molecular ion [M-H]− at m/z 599 (Fig. 5). Daughter ion analysis of this ion gave fragments at m/z 363 and 499 that are not observed in the same analysis of ouabain.

Purification of the Sodium Pump Inhibitor B—This type of sodium pump inhibitor was purified after extraction of 20 kg of bovine adrenals in 11 successive steps including seven HPLC chromatographic stages. Fig. 6 gives an overview of the purification chromatograms. The fraction with a retention time of 70–100 min (first HPLC chromatography) showed a sharp peak in the UV at 220 nm and a high concentration of sodium pump inhibitory material. Therefore, this fraction was used for further purification. The fractions with a retention time of 15–70 min showed a small degree of sodium pump inhibition. When this combined fraction was passed through a Eurocell ONB-carbonate A column containing antibodies against proscillaridin A, the inhibitor A could be purified by affinity chromatography (not shown). The purification of inhibitor B proceeded via six additional reversed phase HPLC steps using varying methanol gradients and resins. From the last chromatographic step, 20 mg of a pure inhibitor B with a retention time and UV spectrum (maximum at 220 nm) identical with ouabain was obtained (Fig. 6, seventh chromatography). Hence, inhibitor B was purified 106 times. Evidently, inhibitor B differed in its properties from inhibitor A.

Properties of the Sodium Pump Inhibitor B from Bovine Adrenals—The purified inhibitor inhibited half-maximally "Rb" uptake into human red blood cells with the same affinity as ouabain (I50 = 4.3 ± 1.2 × 10−8 M) (Fig. 7A). Like ouabain, inhibitor B was unable to inhibit the sodium pump of rat erythrocytes (Fig. 8B). It interacted with antibodies against ouabain but not with antibodies against proscillaridin A (Fig. 8). Moreover, negative ion ESI mass spectral data showed that it had a mass of 584 Da and gave a fragmentation pattern in the daughter ion MS-MS spectrum of the deprotonated molec-
ular ion identical with those of ouabain (Fig. 9). The implication that the sodium pump inhibitor B from bovine adrenals is a compound similar to or identical with ouabain was further strengthened from high field one-dimensional $^1$H NMR data. The well resolved $^1$H spectrum of both compounds has the same signal positions and forms over the whole spectrum (Fig. 10, top). In particular, various signals characteristic of protons in different regions of the molecule can be readily identified and imply identical environments in both molecules. Thus, signals of the single olefinic proton of the lactone ring H-22 (5.96 ppm) coupled to the AB methylene of the same system (5.05, 4.95 ppm), the AB signals of the methylene group at C-19 (4.44, 4.17 ppm), the doublet signal of the methyl group of the rhamnose moiety (1.30 ppm), the tertiary methyl group at C-18 (0.99 ppm), and the complex of signals in the range 1.0–2.4 ppm (Fig. 10, bottom) were similar in both cases. It is reasonable to assume that an isomer of ouabain (7) would show differences in most of these regions. Taken together, the data provide strong evidence for the identity of ouabain and sodium pump inhibitor B.

**DISCUSSION**

Adrenals have previously been shown to be a good source for the isolation of inhibitors of the sodium pump (14, 16). It is evident from the above studies that bovine adrenals contain two different inhibitors. However, neither of these is identical
with the one described by Tamura et al. as adrexin C (14, 16). Although this compound showed properties similar to ouabain, its molecular weight differed. Inhibitor B, which cross-reacted with antibodies against the cardenolide ouabain was purified 10 times from bovine adrenals. According to the $^1H$ NMR and ESI-MS spectral data, it is indistinguishable from ouabain (Figs. 9 and 10). Even high resolution $^1H$ NMR did not reveal any significant differences from authentic ouabain (Fig. 10). This conclusion is also supported by the biological assays. Inhibitor B inhibited the sodium pumps of human and rat erythrocytes like ouabain and interacted with anti-ouabain antibodies (Figs. 7 and 8). This finding is in contrast to the behavior of the ouabain isomer found in the hypothalamus. It binds with a much higher affinity to the $\alpha_1$ isoenzyme of the rat than the plant-derived ouabain (25). Hence, all available data support the conclusion that ouabain and not its isomer was extracted from bovine adrenals. This finding is puzzling insofar as it was reported that both the ouabain-like substances from human plasma and bovine hypothalamus are isomers of ouabain (7); this would therefore suggest that bovine plasma may also contain the ouabain isomer as well, although this has not been confirmed to date. Whether bovine adrenals secrete ouabain or its isomer into the plasma will be of considerable interest. From the finding that ouabain-immunoreactive material is released from bovine adrenal cortical cells in tissue culture by ACTH it is reasonable to suggest that bovine plasma may also contain isomer B from bovine adrenals. This finding is inconsistent with hitherto known cardiotonic steroids; it is also unlikely that the more hydrophilic inhibitor A is a degradation product of ouabain (inhibitor B). Inhibitor A resembles in its retention time and immunological properties the substance whose concentration was found elevated in humans with increased systolic blood pressure (but not diastolic blood pressure) and hence increased pulse pressure (20). Apparently, the type of action of inhibitor A differs from that of ouabain. It is unclear at the present time why several inhibitors of the sodium pump with cardiac glycoside-like action exist (17–19). Do they address different target isoforms of Na$^+$/K$^+$-ATPase in different target cells?

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