V. R. Spiehler,[1] *Ph.D.; Paul Sedgwick,*[1] *B.S.; and R. G. Richards,*[1] *M.D.*

The Use of Brain Digoxin Concentrations to Confirm Blood Digoxin Concentrations

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ABSTRACT: Recent research suggests that the cardiotoxic as well as the neurotoxic effects of digitalis may be mediated by the central nervous system. Therefore brain regions implicated in the genesis of cardiac rhythm disorders were assayed for digoxin. An ^{125}I-labeled radioimmunoassay was used to determine blood and tissue digoxin concentrations. Digoxin was found in the optic tract and optic chiasm in each of four persons who had been taking digoxin regularly. Digoxin is apparently concentrated from blood by the choroid plexus of the fourth ventricle but not by the choroid plexus of the lateral ventricle. However, digoxin was present in the area postrema and nucleus of the vagus only in the two digoxin overdose cases. Digoxin was not detected in any of the other brain regions analyzed. The presence of digoxin in the area postrema (the chemoreceptor trigger zone) and the nucleus of the vagus in the toxic but not in the therapeutic cases suggests a mechanism for the emesis and cardiac arrest brought about by digoxin toxicity in humans. The digoxin content of the medulla, especially the surface of the medulla under the obex, may be useful in confirmation of elevated blood digoxin concentrations.

KEYWORDS: toxicology, digoxin, radioimmunoassay, toxic concentrations, brain concentrations

In coroners' cases in which digoxin has been prescribed for the deceased or in which there is an indication that digoxin overdose may have occurred, blood digoxin levels are quantitated in our laboratory [*1*]. When blood concentrations are found to be greater than 3 ng/mL with symptoms of digoxin toxicity, such as visual disturbance, nausea, vomiting, or cardiac arrhythmias, then the body distribution of digoxin is determined. Since 1976 we have analyzed more than 140 digoxin cases. Of these about 44 cases contained therapeutic blood concentrations of digoxin (between 0.5 and 3.0 ng/mL), 19 cases revealed greater than therapeutic concentrations, and the remainder had no detectable digoxin [*2*].

Since heart blood digoxin concentrations have been reported to increase after death [*3,4*], we investigated the possibility of confirming these values. However, our body distribution studies suggested that most organ digoxin concentrations, with the exception of the liver, may not be useful in confirming blood digoxin concentrations [*2*]. Surprisingly, the heart digoxin

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[1] Toxicologist, toxicologist, and pathologist, respectively, Office of the Sheriff-Coroner, Orange County, Santa Ana, Calif.

concentrations we found in digoxin overdose cases were not different from those reported after therapeutic digoxin administration [*2,5-7*].

An increasing amount of research data suggests that both the cardiac and the noncardiac toxic effects of digitalis may be mediated through the central nervous system [*8*]. Neurotoxicity as manifest by anorexia, nausea, blurred and yellow-green vision, and vomiting has been used as a sign of impending cardiotoxicity. Psychoses, sleep disturbances, hallucinations, and delirium have also been reported in digoxin intoxication [*9-11*]. Recently the central nervous system has also been implicated in the genesis of cardiac rhythm disorders [*8,12-14*]. These studies have pointed to the involvement of two specific brain regions in the neurally mediated arrhythmogenic properties of digitalis: the ventral medial nucleus of the hypothalamus and the dorsal surface of the medulla under the obex. Therefore, we investigated local digoxin concentrations in these and adjacent brain regions in some recent coroner's cases involving digoxin.

Methods

Approximately 150 mL of heart blood was collected in a screw-cap glass bottle with 0.75 g potassium oxalate and 2.0 g sodium fluoride. Prior to analysis the jar was gently inverted and duplicate 100-μL samples were removed for analysis. The whole brain was removed at autopsy and placed in double plastic bags. Brains were refrigerated until dissection. Upon dissection, brain tissue was placed in screw-capped glass jars, labeled, and frozen at $-160°C$ without preservative until analysis. Solid tissue was weighed and homogenized with its weight of normal saline with 5% methanol in an Eberach micro-grinder on a commercial Waring motor at high speed for approximately 1 min.

For dissection the whole brain was inverted in a glass dish. The optic nerves up to the optic chiasm were removed and combined. The optic chiasm was then removed. Then each optic tract was cleaned and removed and the two were combined. The lateral geniculate nuclei were located at the entry of optic tract into the thalamus, removed, and combined with each other. At this step the choroid plexus of the lateral ventricle is usually exposed. These plexuses were removed with forceps and combined. Then the ventral-medial nucleus of the hypothalamus was removed.

The brain was then righted and the cerebellum removed. Cerebellar tissue directly above the fourth ventricle was sampled. The choroid plexus of the fourth ventricle was removed. Then the surface (4 to 5 mm deep) of the medulla just above the obex was excised. The remainder of the medulla was sampled. Samples of prefrontal cortex and visual cortex were also taken. Samples were homogenized with an equal volume of normal saline with methanol (0.85% w/v sodium chloride with 5% methanol).

Digoxin Analysis

The Beckman Solid Phase Digoxin Reagent System® was used for quantitation. This radioimmunoassay used ^{125}I-labeled digoxin as the tracer, rabbit anti-digoxin antibody as the specific binding reagent, and goat anti-rabbit gamma globulin as a specific precipitating antibody for separation of bound and free radiolabeled tracer. The assay protocol as specified by the manufacturer was not modified. Whole blood and brain homogenates were assayed directly without extraction. After being centrifuged in a Beckman Microfuge B® and decanted, the bound fraction was counted for 1 min on a Picker Pace 1® system.

All samples with an apparent digoxin concentration greater than the highest standard (6 ng/mL) were diluted appropriately with normal saline (0.85% sodium chloride with 5% methanol). Each dilution was then reanalyzed in duplicate to give a value within the range of the standards.

Results

Case 1

The deceased, a 60-year-old female, had taken 40 to 50 Lanoxin® tablets at about 5:00 p.m. She was admitted to the hospital at 9:00 p.m. and given 25 mg Dramamine® intramuscularly for retching; 100 mg lidocaine intravenously and 2 g lidocaine in an intravenous drip; and 100 mg Dilantin® (three times). She was defibrillated eleven times in the early morning hours. The patient died (complete heart block) at 6:25 a.m. The autopsy was performed the afternoon of the same day.

The brain and blood digoxin concentrations are shown in Fig. 1. The cardiac glycoside digoxin does not pass the blood-brain barrier because of the sugar portions of its structure. However, it is apparently concentrated from blood by the choroid plexus of the fourth ventricle. Cerebrospinal fluid is manufactured from blood at the choroid plexuses of both the lateral ventricles and the fourth ventricle. However, the choroid plexuses of the lateral ventricles appear to have a digoxin content like that of the blood, in contrast to the choroid plexus of the fourth ventricle, which appears to concentrate digoxin two to three times the values found for heart blood (Table 1). The choroid retina of the eye also concentrates digoxin [*15*].

The highest brain concentration was found in the area postrema and nucleus of the vagus dissected from the floor of the fourth ventricle at the obex. On the other hand, no digoxin was detected in the control samples from nearby areas of the cerebellum and the remainder of the medulla. These tissue digoxin concentrations are consistent with the retching of the deceased noted and treated on admission to the hospital. Stimulation of the area postrema, the chemoreceptor trigger zone, produces nausea and vomiting. Borison and Wong [*16*] showed that the area postrema mediates the emetic effects of digitalis. Therefore, the finding of high

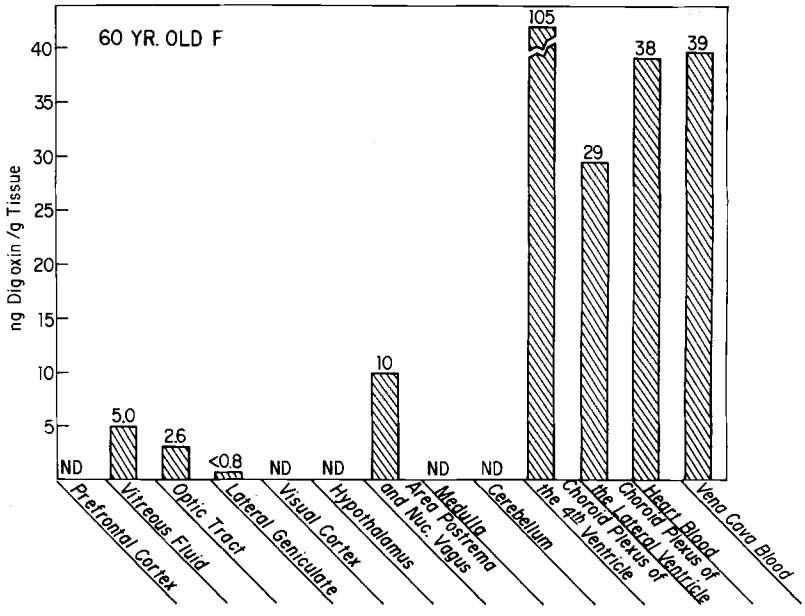

FIG. 1—*Regional brain digoxin concentrations in an overdose case.*

TABLE 1—*Regional brain digoxin concentrations (ng/g or ng/mL).*[a]

Case	Blood	Optic Chiasm	Optic Tract	Lateral Geniculate Nucleus	Area Postrema	Choroid Plexus	
						Lateral Ventricle	Fourth Ventricle
4	1.3	4.7	2.2	0	0	1.1	2.2
3	1.6	0.8	0	0	0	3.5	...
2	6.8	3.3	3.5	0	1.4	6.2	16.2
1	31.0	(5.0)[b]	2.6	0.8	10.0	38.0	105.0

[a]No digoxin was found in any case in the visual cortex, hypothalamus, medulla, cerebellum, or prefrontal cortex.
[b]Vitreous humor.

tissue concentrations of digoxin in the area postrema is consistent with the observed violent vomiting in this case.

Somberg and Smith [*14*] have described experiments in cats indicating that the locus of digitalis-induced cardiac arrhythmias is an area in the medulla within 2 mm of the obex. The nucleus of the vagus nerve as well as the area postrema is contained in the tissue dissected from this region of the obex. In Case 1 this tissue contained 10 ng digoxin per gram. Basu-Ray and Pradhan [*17*] demonstrated that microinjection of ouabain into the dorsal nucleus of the vagus or the nucleus tractus solitarius results in sinus bradycardia, junctional rhythm, ventricular tachycardia, and death by cardiac arrest. Therefore the arrhythmias and cardiac arrest observed in Case 1 may be due to the entry of digoxin into the nucleus of the vagus nerve. If this is the case, no digoxin would be found in this region in the brains of persons receiving digoxin therapeutically who died of other causes. Investigation of the regional brain distribution of digoxin in several therapeutic cases suggests that this may be so (Table 1, Cases 3 and 4).

Case 2

The deceased, a 75-year-old female, was admitted to the hospital with a fractured left shoulder and hip. She had been prescribed 0.25 mg digoxin a day and had a serum digoxin concentration of 1.6 ng/mL when checked two months before admission to the hospital. Her serum digoxin level on admission to the hospital was 0.5 ng/mL. She suffered a cardiac arrest during her hospital stay and was placed in the cardiac intensive care unit but died during a second cardiac arrest.

Case 3

The deceased was an 85-year-old female who fell while getting out of bed. A computerized axial tomography (CAT) scan revealed a subdural hematoma, which was removed by emergency surgery. However, she began having seizures and died the following day. She had been taking 0.125 mg Lanoxin daily.

Case 4

The deceased, a 65-year-old male, collapsed suddenly at home and could not be revived by the fire department paramedics who were called. He had retired five years earlier because of emphysema and heart disease. He had taken 0.25 mg digoxin/day for the five years.

Discussion

In Cases 3 and 4 blood concentrations were consistent with therapeutic concentrations of digoxin. In these cases no digoxin was found in the area postrema. However, in every case digoxin was found in the optic chiasm and optic tract. DiMaio et al [*18*] reported that the concentration of digoxin in vitreous humor equilibrates with that in blood, although slowly. Binnion and Frazer [*15*] have shown that digoxin concentrations in vitreous humor increase after death. The presence of digoxin in the optic chiasm and optic tract may be due to diffusion from the vitreous humor along the optic nerve up to the synapse in the lateral geniculate nucleus of the thalamus. We did not find digoxin in the visual cortex in any case. Digoxin was present in trace amounts in the lateral geniculate nucleus in Case 1 but not in Cases 2 to 4. Disturbances of vision have long been recognized as the earliest symptom of digitalis toxicity [*19,20*]. In addition, the occurrence of bright-colored visual hallucination has been correlated with serum digoxin concentrations of 3 to 7 ng/mL [*15*].

Digoxin was not found in any case in the prefrontal cortex, medulla, cerebellum, visual cortex, or the hypothalamus. The failure to find digoxin in the hypothalamus suggests that, at least in this case, atrial fibrillation caused by hypothalamic sympathetic discharge was not the mechanism of digoxin toxicity.

Summary

The highly polar cardiac glycosides do not cross the blood brain barrier. However, digoxin may enter the brain by two routes. In the first, blood digoxin slowly equilibrates with vitreous humor. In all cases studied digoxin was found in the optic nerve, optic chiasm, and optic tract. The presence of digoxin in the optic system is evidence of chronic doses. Its presence in the lateral geniculate nucleus may relate to digoxin's well-known effects on visual perception.

The second point of entry into the central nervous system appears to be at the brain stem. Digoxin is concentrated from blood by the choroid plexus of the fourth ventricle. Measurable digoxin concentrations were found in the tissue under the obex in both cases where death was accompanied by vomiting and cardiac arrest. The presence of digoxin in the area postrema (the chemoreceptor trigger zone) and the nucleus of the vagus in the toxic but not in the therapeutic cases described above suggests a mechanism for the emesis and cardiac arrest of digoxin toxicity in humans. Therefore the digoxin content of the medulla, especially the surface of the medulla under the obex, may be useful in the confirmation of elevated blood digoxin concentrations and in the confirmation of digoxin involvement in death.

These conclusions are tentative because of the small number of cases. Our study of brain digoxin concentrations is continuing.

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Address requests for reprints or additional information to
Vina R. Spiehler, Ph.D.
Office of the Sheriff-Coroner, Orange County
P.O. Box 449
Santa Ana, Calif. 92702