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ACCUMULATION OF ^3H -DIGITOXIN IN CNS OF CATS WITH A HYPERSENSITIVITY TO THE DRUG

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Considerable literature (1-6) is available on "the cumulative effect of digitalis", but the mechanism involved in the appearance of this "cumulative effect" is still a matter of speculation. Previous reports suggest the possibility that cardiac glycosides may produce their effects on the heart through the central nervous system (CNS) (7, 8). The present work was designed to determine whether "the cumulative effect of digitalis" occurs as a result of the central accumulation of digitalis or is due to the cardiac accumulation of the drug.

The experiment was carried out using cats. At both one and 48 hours after a single injection ($17\text{ }\mu\text{Ci}/132\text{ }\mu\text{g/kg}$) of ^3H -digitoxin (New England Nuclear Corp., S.A.: 26 mCi/mg), the following procedures were carried out; 1) determination of both arrhythmic and lethal doses of digitoxin by Hatcher's method, 2) measurement of glycoside (digitoxin and its metabolites) content in the medulla, midbrain, heart, kidney and liver by means of the combustion method (Tri-Carb sample oxidizer, Packard Model 305). Radioactivity was determined using a liquid scintillation spectrometer. CHCl_3 soluble extracts were separated by a thin layer chromatographic technique after a CHCl_3 -extraction from the homogenate of the each tissue.

At 48 hours after the injection, both arrhythmic dose ($190 \pm 25\text{ }\mu\text{g/kg}$) and lethal dose ($317 \pm 33\text{ }\mu\text{g/kg}$) of digitoxin were decreased to one half of each control value (arrhythmic dose; $423 \pm 45\text{ }\mu\text{g/kg}$, lethal dose; $660 \pm 43\text{ }\mu\text{g/kg}$). At one hour after the injection, both arrhythmic and lethal doses were found to be much the same as the control values. Forty-eight hours after the injection of digitoxin, a marked hypersensitivity to the drug was seen

FIG. 1. Thin layer radiochromatograms of the chloroform extracts of serum, midbrain and heart after intravenous administration of ^3H -digitoxin to cats. The determination was carried out at 48 hours after the administration of ^3H -digitoxin ($17\ \mu\text{Ci}/132\ \mu\text{g/kg}$). Solvent system; methylethylketon, xylene, chloroform (2:2:1). SL=start line, SF=solvent front, A=digoxin, B=digitoxin, C=digoxigenin, D=digitoxigenin.

in the cats, but one hour after the injection the cats showed no such hypersensitivity to this drug. One hour and 48 hours after the injection of ^3H -digitoxin, digitoxin and its metabolites were measured. At 48 hours after the injection the percent of digitoxin to the total radioactivity was as follows, blood (30.7), medulla (78.31), midbrain (75.08), heart (67.40), kidney (93.85) and liver (55.98). It was ascertained on thin layer chromatogram that radioactivity in CHCl_3 -extracts represented digitoxin only. In addition, water soluble metabolites represented digitoxin conjugates that were identified after this fraction was extracted with CHCl_3 following heating under a condition of $1/10\ \text{N HCl}$. Therefore, the radioactivity obtained was calculated as digitoxin. Fig. 1 shows typical thin layer chromatograms of the CHCl_3 -extracts of blood, the midbrain and the heart. Radioactivity is shown as only one spot, and this spot represents digitoxin. Table 1 shows the total radioactivity in various tissues 1 hour and 48 hours after the injection. The concentration of digitoxin in the medulla and midbrain at 1 hour (A) after the injection was lower than the concentration in the blood.

TABLE 1. Concentration of total digitoxin in tissues 1 hour and 48 hours after intravenous administration of ^3H -digitoxin to cats

Tissue	Total digitoxin (CHCl_3 soluble + Water soluble) $\mu\text{g/g}$ tissue		ratio B/A
	1 hr (A)	48 hr (B)	
Serum	0.182 ± 0.041	0.055 ± 0.010	0.30
Midbrain	0.063 ± 0.016	0.154 ± 0.018	2.44
Medulla	0.077 ± 0.023	0.138 ± 0.016	1.79
Heart	0.639 ± 0.122	0.180 ± 0.020	0.28
Liver	2.936 ± 0.577	0.291 ± 0.078	0.10
Kidney	5.520 ± 1.302	1.033 ± 0.260	0.19

These data are in good agreement with the results reported by Weaver et al. (9) and Dutta et al (10). As shown in the table, the total digitoxin concentration of both blood and the heart decreased to one third at 48 hours after the injection compared to the value at 1 hour after the injection. On the contrary, the content of digitoxin in the medulla and the midbrain markedly increased.

It is a very important finding that these values in the medulla and midbrain were markedly higher than values in the blood at 48 hours. The calculated molar concentrations of digitoxin in the medulla, midbrain and heart at 48 hours after the injection were $0.15 \mu\text{M}$, $0.14 \mu\text{M}$ and $0.15 \mu\text{M}$. The activity of Na-K ATPase extracted from the cell membrane of cat cardiac muscles was inhibited by 38% against the control value by $0.18 \mu\text{M}$ digitoxin (11). Therefore, when both the medulla and midbrain contain digitoxin, a hypersensitivity to the drug may ensue.

From the present results using cats, it can be deduced that central mediated hypersensitivity to digitoxin is present as a result of the cumulative effect of the drug.

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