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ACCUMULATION OF RADIOACTIVE CARDIAC GLYCOSIDES BY VARIOUS BRAIN REGIONS IN RELATION TO THE DYSRHYTHMOGENIC EFFECT

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1 Ouabain was administered at a loading dose of 3 μg/kg followed by an infusion at a rate of 1 μg kg^{-1} min^{-1} in order to produce severe dysrhythmia in dogs within 60 minutes. Similarly, digitoxin at a loading dose of 9 μg/kg followed by an infusion at a rate of 3 μg kg^{-1} min^{-1} was administered to compare its effect with that of ouabain.

2 During the 60 min experimental period, the plasma concentrations gradually rose with the continuous infusion of these drugs. However, in comparison to the 60 min plasma value of 119 ± 20 pmol/ml for ouabain and 177 ± 68 pmol/ml for digitoxin, the cerebrospinal fluid (CSF) concentrations for these drugs at this time were less than 5 pmol/ml.

3 Upon termination of the experiment at 60 min it was found that kidney, liver, heart, adrenal, and the non-neural tissue in the brain such as pituitary and choroid plexus concentrated ouabain and digitoxin to give high tissue to plasma ratios. However, various neural areas of the brain (cerebellum, mesencephalon, hypothalamus, pons, and medulla) showed no preferential localization or uptake of these two glycosides.

4 Concentration of ouabain and digitoxin by the choroid plexus does not seem to affect the ionic composition of the CSF.

5 It was concluded that sampling the large areas of neural tissue listed above could provide no evidence for local accumulation of digitalis glycosides that might account for a central nervous system origin of digitalis-induced cardiac arrhythmias.

Introduction

Previous reports have drawn attention to the possibility that cardiac glycosides may produce effects on the heart through the central nervous system (CNS), apart from any direct action upon the myocardium. Recently, results implicating the CNS in the dysrhythmogenic effects of cardiac glycosides have been reported by Gillis and colleagues (Gillis, 1969; Gillis, Dionne & Standaert, 1972a; Gillis, Raines, Sohn, Levitt & Standaert, 1972b). By simultaneously recording sympathetic and phrenic nerve activity together with the ECG, the above workers demonstrated a relationship between the enhanced neuronal activity and cardiac disorders following systemic injection of digitalis. We felt that these studies of Gillis (1969) and recent claims of Garan, Smith & Powell (1974) were sufficiently impressive to require careful evaluation of the CNS as a possible site of digitalis-induced dysrhythmia. Thus, we undertook a systematic evaluation to determine: (1) the rate of appearance of systemically administered cardiac glycosides, namely ouabain and digitoxin, in the cerebrospinal fluid (CSF); (2) the uptake of these cardiac glycosides in various brain areas; and (3) the effect of these cardiac glycosides upon the ionic composition of the CSF.

Methods

Experiments were conducted on male and female mongrel dogs anaesthetized with a loading dose of 1 ml/kg α-chloralose (7.5%) and urethane (50%). The anaesthesia was supplemented, if necessary, by 0.1 ml/kg doses of this solution. Cardiovascular measurements including left intraventricular pressure (LVP), the rate of change of LVP (dp/dt), aortic blood pressure and lead II ECG were continuously monitored on a Beckman dynograph. The LVP was recorded by retrograde insertion of a catheter through the left femoral artery into the lumen of the left ventricle. The end of the catheter was connected to a Beckman miniature pressure transducer and the ventricular pressure wave was recorded on one channel of the polygraph. The first derivative of the output from the LVP was obtained directly from the ventricular pressure signal by means of a resistance-

capacitance (R-C) differentiating circuit and displayed on another channel of the polygraph. Aortic pressure was recorded in the thoracic aorta by inserting another catheter through the right femoral artery. This catheter was also connected to a Beckman pressure transducer by means of a three-way stop-cock.

Arterial blood samples were collected serially through the aortic catheter. A 22 gauge spinal needle was inserted into the cisterna magna for serial collection of CSF samples. After control samples of blood and CSF were taken, tritium labelled ouabain or digitoxin was administered intravenously. The dose of ouabain consisted of an injection of a 3 μg/kg loading dose followed by infusion at a rate of 1 μg kg^{-1} min^{-1}. In the case of digitoxin, a loading dose of 9 μg/kg was followed by a 3 μg kg^{-1} min^{-1} infusion. Unlabelled ouabain and digitoxin were mixed with small amounts of tritiated ouabain and digitoxin of high specific activity. Depending on the weight of the dog, approximately 50–60 μCi of tritiated drug was administered per experiment. Radioactive ouabain (20 μCi/μg) and digitoxin (40 μCi/μg) were obtained commercially as the randomly labelled product (New England Nuclear Corp.). Drugs were infused with a Desaga peristaltic pump through a catheter inserted into the left femoral vein.

At the termination of each experiment, 30–50 mg samples from left ventricle of the heart, kidney, liver, and adrenal gland were obtained along with brain samples to determine their radioactive content. The brain areas dissected out for radioactive counting were: medulla, pons, mesencephalon, cerebellum, visual cortex, hypothalamus, pituitary, and the choroid plexus of the lateral ventricles. Radioactivity in the tissue, blood plasma, and CSF samples was determined by a liquid scintillation spectrometer. Commercially available phase combining system (Amersham–Searle) was used as the scintillation fluid. All samples were counted for a period of time sufficient to limit the counting error to 5% or less.

For measurement of the plasma and CSF sodium, potassium, calcium, and magnesium, aliquots were analyzed with an atomic absorption spectrophotometer. The concentration of each ion was expressed in mmol/litre of the respective body fluid.

Results

Electrophysiological effects manifested by dysrhythmogenic doses of cardiac glycosides

In all dogs treated with ouabain, severe ventricular tachycardia was observed within 60 min, at which time the experiment was terminated. In some experiments, dysrhythmia appeared after 15 min of infusion and was sustained throughout but in others, the onset was later. In contrast, digitoxin did not induce severe dysrhythmia during the one hour infusion period despite the dosage being three times as great as that of ouabain.

Plasma and CSF cardiac glycoside concentration

After administration of the loading dose and initiation of ouabain and digitoxin infusion, plasma concentrations of the drugs gradually rose but did not plateau during the 60 min experimental period. The average plasma and CSF concentrations of ouabain at 60 min were 119 ± 20 and 2.9 ± 1.2 pmol/ml, respectively (Tables 1 and 2). Digitoxin plasma levels were somewhat higher, but the CSF concentration of digitoxin was similar to that of ouabain. The plasma/CSF ratio for ouabain and digitoxin showed no significant difference and appeared to stabilize at approximately 40:1 and 50:1 respectively.

Ouabain and digitoxin concentration in brain regions and in other tissues

No neuronal brain region studied, including the hypothalamus, pons, and medulla was found to

Table 1 The concentrations of [^{3}H]-ouabain and [^{3}H]-digitoxin in plasma and cerebrospinal fluid (CSF) at various time intervals following intravenous administration of these drugs

	Plasma (pmol/ml)		*CSF (pmol/ml)*	
	Ouabain	*Digitoxin*	*Ouabain*	*Digitoxin*
Time (min)	*mean ± s.e.* (n=6)	(n=3)	(n=6)	(n=3)
5	46 ± 9	54 ± 15	—	0.8 ± 0.7
10	50 ± 12	—	0.9 ± 1.0	—
15	—	88 ± 27	—	0.5 ± 0.5
20	65 ± 15	—	1.2 ± 1.3	—
30	68 ± 12	121 ± 36	2.3 ± 2.8	2.0 ± 1.4
40	93 ± 18	—	2.1 ± 1.1	—
45	—	152 ± 52	—	2.4 ± 1.5
60	119 ± 20	177 ± 68	2.9 ± 1.2	3.2 ± 3.2

contain more than a small amount of ouabain. The ouabain content was 10% or less of the plasma concentration (Table 2). However, the pituitary and choroid plexus, concentrated ouabain to a great extent as reflected by 7.1 and 1.74 fold tissue: plasma binding ratios. Localization of ouabain in the visceral organs and skeletal muscle was generally high, the kidney accumulating ouabain to the greatest extent. The heart also accumulated a substantial concentration of ouabain and lesser levels were found in the adrenal gland and liver. Skeletal muscle accumulated the least on a weight basis.

Although there were small differences between the ouabain and digitoxin concentrations in the tissues

Table 2 The concentrations of [^{3}H]-ouabain and [^{3}H]-digitoxin in the visceral and brain tissue at 60 min after intravenous administration of these drugs

Tissue	*Ouabain (n=6) (pmol/g wet weight) mean ± s.e.*	*Digitoxin (n=3) (pmol/g wet weight) mean ± s.e.*
Heart	432 ± 110	190 ± 33
Kidney	3219 ± 1121	882 ± 212
Liver	250 ± 125	383 ± 187
Adrenal	269 ± 147	235 ± 133
Skeletal muscle	56 ± 29	73 ± 28
Pituitary	845 ± 193	256 ± 137
Choroid plexus	204 ± 59	670 ± 124
Cerebellum	11 ± 5	26 ± 21
Hypothalamus	12 ± 6	24 ± 17
Pons	9 ± 6	18 ± 13
Medulla	12 ± 7	19 ± 14
Mesencephalon	10 ± 6	21 ± 10
Plasma	119 ± 20	177 ± 68
CSF	2.9 ± 1.2	3.2 ± 3.2

Table 3 The potassium concentration (mEq/ml) of the plasma and cerebrospinal fluid at various times after intravenous administration of saline (control), ouabain, digitoxin, and KCl (1.0 mEq kg^{-1} h^{-1})

Time (min)		0	5	15	30	45	60
Control	Plasma	3.94 ± 0.16 (3)*	4.03 ± 0.04 (3)	3.83 ± 0.30 (3)	3.95 ± 0.25 (3)	3.97 ± 0.21 (3)	4.02 ± 0.17 (3)
	CSF	3.33 ± 0.17 (3)	3.25 ± 0.16 (3)	3.22 ± 0.18 (3)	3.21 ± 0.12 (3)	3.23 ± 0.09 (3)	3.18 ± 0.04 (3)
Ouabain	Plasma	4.04 ± 0.62 (5)	4.23 ± 0.78 (5)	4.65 ± 0.79† (5)	5.10 ± 0.84† (5)	6.03 ± 1.34† (5)	5.66 ± 0.84† (4)
	CSF	3.05 ± 0.21 (4)	3.17 ± 0.26 (4)	3.14 ± 0.17 (4)	3.08 ± 0.22 (4)	3.22 ± 0.22 (3)	3.03 (2)
Digitoxin	Plasma	3.87 ± 0.51 (3)	3.88 ± 0.66 (3)	3.86 ± 0.62 (3)	3.93 ± 0.66 (3)	3.94 ± 0.88 (3)	4.06 ± 0.64 (3)
	CSF	2.72 ± 0.31 (3)	2.82 ± 0.17 (3)	2.82 ± 0.16 (3)	2.76 ± 0.14 (3)	2.80 ± 0.17 (3)	2.81 ± 0.17 (3)
K^+ infusion	Plasma	4.02 ± 0.17 (3)	4.50 ± 0.14 (3)	4.65 ± 0.45 (3)	4.93 ± 0.49† (3)	5.02 ± 0.83† (3)	5.09 ± 0.22† (3)
	CSF	3.18 ± 0.04 (3)	3.31 ± 0.03 (3)	3.32 ± 0.06 (3)	3.20 ± 0.16 (3)	3.38 ± 0.14 (3)	3.30 ± 0.09 (3)

* Number of experiments in parentheses throughout Table.
† Significantly higher than the value at 0 time: $P < 0.05$.

examined, the general pattern of their distribution was similar (Table 2). It is noteworthy that as with ouabain, there was negligible localization of digitoxin in the neural tissue whereas choroid plexus and pituitary concentrated digitoxin to a great extent. Neural tissue concentration of digitoxin was in general less than 15% of the plasma concentrations.

Ionic composition of plasma and CSF as affected by arrhythmogenic doses of cardiac glycosides

The concentrations of potassium, sodium, calcium, and magnesium were measured in plasma and CSF at different times following ouabain and digitoxin infusion. In general, the plasma concentrations of sodium, calcium, and magnesium remained at control levels of 140, 4.0, and 1.0 mEq/litre respectively after the administration of the cardiac glycosides. However, during the 60 min experimental period of ouabain treatment, the plasma $[K^+]$ gradually increased from the control level to a level of about 6 mEq/litre while the potassium levels in the CSF remained close to those of the control values (Table 3). The absence of change in CSF $[K^+]$ at a time when plasma $[K^+]$ was elevated was also seen in a separate series of experiments in which the plasma $[K^+]$ was raised by the infusion of 0.9% w/v NaCl solution (saline) containing KCl at a rate of 1.0 mEq K^+ kg^{-1} h^{-1} (Table 3). Thus, it appears that neither ouabain nor the potassium infusion altered CSF $[K^+]$ concentration. In the experiments with digitoxin, unlike those in which ouabain was used, neither the plasma nor CSF levels of $[K^+]$ changed.

Discussion

The results presented in this study clearly indicate that no specific localization of ouabain or digitoxin occurred in any CNS tissue examined. Furthermore, both glycosides apparently remained within the extracellular space of the brain and did not appear to be generally available for interaction with the neural tissue. Using the terminal plasma level of 118.6 pmol/ml obtained here for ouabain, a substance known to show no significant protein binding (Kuschinsky, 1968), it would be expected that 21 pmol of ouabain would be present in a gram of the tissue due to distribution in the extracellular space, assuming that the extracellular space in brain tissue is approximately 18% (Levin, Fenstermacher & Patlak, 1970). However, all of the brain regions sampled contained substantially smaller amounts of ouabain than the amount that could be accounted for in the extracellular space. Although the lack of accumulation of ouabain by the brain was understandable in terms of physio-chemical limitations of this highly polar compound to penetrate lipid barriers, the low concentration of digitoxin found in the neural tissue was at first unexpected, since digitoxin is exceedingly lipid soluble. However, since as much as 90–95% of the plasma digitoxin may be albumin bound (Kuschinsky, 1968; Lukas, 1971), it might be expected that less than 10 pmol/ml of digitoxin would be available for diffusion from plasma into the brain. On the basis of this amount of free digitoxin present in the plasma, the various brain areas accumulated digitoxin at least 10 times more readily than ouabain, although the absolute tissue: plasma ratios were only slightly higher. No brain region examined showed any selective or unusual digitoxin uptake, although the absence of dysrhythmia with this dose of digitoxin and the possible slower equilibration of digitoxin leaves open the possibility of delayed brain localization of this drug.

The very low CSF/plasma ratio (0.01–0.03) of these cardiac glycosides further indicates that these agents do not easily penetrate the blood brain barrier. Recently, Garan *et al.* (1974) determined the CSF and plasma concentrations of digoxin following intravenous injection. They found that the highest CSF value was 2.3 ng/ml (2.9 pmol/ml) with a CSF/plasma ratio of about 0.05. Although they claimed that the highest value of digoxin in CSF, attained at 15 min, corresponded with the peak coronary vascular effect, in our study the peak value did not correspond to particular physiological correlates. However, they used a bolus injection rather than a loading dose and continuous infusion, so that some differences in time course might be expected.

No specific localization of ouabain occurred in any of the neural areas examined and the mechanism by which ouabain facilitated sympathetic discharge in the hands of Gillis and co-workers (Gillis, 1969; Gillis *et al.*, 1972a & b) remains in doubt. It would be easier to accept a CNS origin of ouabain-induced dysrhythmia if some region of the brain exhibited adequate ouabain-accumulating activity to account for this response. Intracerebral injections of ouabain to produce ventricular dysrhythmia require a cumulative dose of 100 μg (Basu-Ray, Booker, Dutta & Pradhan, 1972; Saxena & Bhargava, 1975), an amount much greater than the amounts of ouabain per g of tissue found in the various dog brain regions in the present experiments. Of course, it is possible that the appropriate brain region was not sampled, although regions previously implicated were included, and it is also possible that a high local concentration in part of the brain escaped detection. Both of these possibilities deserve further study, but for the time being, a CNS site of action for ouabain-induced dysrhythmia is not supported by our results. An alternative, more plausible explanation may implicate peripheral neural structures, including afferents and ganglionic synapses.

Perhaps the site of action of these drugs is in some non-neural structure in the brain such as the choroid

plexus or pituitary gland. There is very little information available at present suggesting that the CNS effect of ouabain can be mediated by the pituitary gland. On the other hand, the preferential accumulation of both ouabain and digitoxin in the choroid plexus suggests the possibility that these drugs may influence the active transport of ions and various amines and/or amine metabolites into the CSF, although we could find no change in cation content of CSF.

CSF has relatively more sodium and magnesium and less potassium and calcium than plasma ultrafiltrate and the reported values for the CSF/plasma concentration ratios of sodium, magnesium, potassium, and calcium are 0.95, 1.1, 0.75, and 0.53, respectively (Pollay, 1974). Using an extracorporeal perfusion technique with sheep choroid plexus, Pollay, Kaplan & Nelson (1973) have recently obtained results indicating that the movement of potassium from plasma to CSF is mediated by the Na-K pump which saturates at a plasma concentration of 3.3 mmol/litre and is inhibited by ouabain (10 μM) in CSF or plasma. The present study shows that ouabain at a free plasma concentration of 0.1 μM did not greatly alter the K^+ concentration in CSF but produced severe arrhythmia.

Although the precise subcellular site of binding for cardiac glycosides in the choroid plexus has yet to be determined, Quinton, Wright & Tormey (1973) have demonstrated by autoradiography that [^{3}H]-ouabain binds to the brush border of the frog ependyma *in vitro*. A recent study on the localization of Na-K ATPase in the choroid plexus of various mammalian species appears to contradict these observations in the dog. Milhorat, Davis & Hammock (1975), using the Ernst technique, found that the ATPase activity is well localized along the basolateral membrane. The contradiction may, of course, only be apparent, for the transport enzyme is not necessarily the sole specific binding site for the drug, and species differences may be important. In the present *in vivo* studies, the drugs may well have bound to the basolateral membrane of choroid plexus initially and subsequently gained access to the brush border after intracellular translocation.

Other workers have recently found preferential accumulation of digoxin in the CSF and choroid plexus of man (Neblett, McNeal, Waltz & Harrison, 1972; Bertler, Anderson & Wettrell, 1973) and dog (DasGupta & Binnion, 1974). These observations made for digoxin compare well with the ouabain and digitoxin accumulation found in the present study. What differences exist for the choroid plexus/plasma concentration ratios, may be understood, in part, in terms of the amount of free drug available in each case. Although it is clear from this study, as well as previous studies (Bertler *et al.*, 1973; DasGupta & Binnion, 1974), that there is a preferential localization of cardic glycoside in the choroid plexus, the pharmacological implication of this binding has yet to be established.

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