Distribution of Anticonvulsant Drugs in Gray and White Matter of Human Brain

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SUMMARY: *Gray and white matter were obtained during neurosurgical therapy of focal epilepsy from 17 patients. In 10 patients, receiving only phenobarbital, the drug was uniformly distributed between gray and white matter. Phenytoin concentrations averaged 1.4-fold greater in white matter than in gray matter when expressed per gram wet weight of tissue. The gray matter/plasma ratio of phenytoin was approximately 2-fold greater than that of phenobarbital. Carbamazepine levels were also slightly greater in white matter The data revealed wide differences between drugs in the relative concentrations in gray and white matter, which must be taken into account in any quantitative studies of anticonvulsant drug levels in the brain.*

RÉSUMÉ. *Nous avons étudié des échantillons de cortex cérébral et de substance blanche obtenus de 17 malades opérés pour une épilepsie focale. Dix d'entre eux ne recevaient que du phénobarbital et la concentration de cette drogue était égale au niveau du cortex et de la substance blanche. Exprimée par gamme de tissue frais, la concentration de phénytoine s'est avérée en moyenne 1.4 fois plus élevée au niveau de la substance blanche que de la matière grise Le rapport matière grise/plasma est environ 2 fois plus grand pour la phénytoine que pour le phénobarbital. La concentration de Carbamazépine est aussi légèrement plus élevée au niveau de la substance blanche. Ces données démontrent des différences marquées entre ces drogues substance blanche. Differences dont on doit tenir compte dans l'interprétation des études neurophysiologiques ou neuropharmacologiques de la concentration cérébrale des drogues antiépileptiques.*

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It has been established that plasma anticonvulsant drug monitoring can help seizure control. The intensity and duration of the therapeutic action of anticonvulsant drugs depend on the maintenance of an adequate concentration of the drug at hypothetical receptor sites, presumably in neuronal membranes. Anticonvulsant activity has been correlated with the concentration of a drug or its active metabolite(s) (Kupferberg and Yonekawa, 1975). Though most of the drug present in the brain is bound non-specifically by various tissue components, the total concentration in the brain presumably reflects the concentration of freely diffusable drug in plasma water and gives an indication of specific binding by putative specific receptor sites.

Previous studies have demonstrated a significant correlation between phenytoin and phenobarbital in cerebral tissue and plasma obtained during the neurosurgical therapy of focal epilepsy. The aim of the present study was to determine the relative distribution of phenytoin, phenobarbital and carbamazepine between the gray matter of human cerebral cortex and subcortical white matter.

METHODS

Seventeen patients were selected from those undergoing neurosurgical treatment of focal epilepsy. In all patients prolonged trials of various anticonvulsant drugs had failed to achieve adequate seizure control. All of the patients studied were at steady-state, having received fixed maintenance doses of the various anticonvulsant drugs for a period of at least 5 half-lives up to the time of operation. Cerebral tissues were ob-

tained immediately following the block removal of the temporal (14 patients) or frontal (3 patients) lobes. The excisions were performed under local anesthesia to permit clinical electrographic localization of functionally critical regions of the cerebral cortex. Tissue surrounding the epileptogenic focus, but by necessity included in the surgical excision, was dissected free and the gray and white matter separated by fine dissection. Tissues were immediately frozen in liquid nitrogen and stored at —20°C. Adjacent portions were submitted to pathological examination. Patients with brain tumors or other structural lesions were excluded from the study. Venous blood was collected in the presence of editic acid (EDTA) at the time of the surgical excision and the plasma separated by centrifugation.

Drug determinations. Duplicate samples of brain weighing approximately 300 mg wet wt. were homogenized in distilled water (20% W/V) and extracted with ethylene dichloride as previously described (Sherwin et al., 1973). Phenytoin and phenobarbital were determined by gas-liquid chromatography employing on-column methylation with an internal standard (5-p-tolyl-)-5-phenylhydantoin (Kupferberg, 1970). The actual recoveries from tissues were: phenobarbital, 90.3%; phenytoin, 83.7%. The coefficients of variation for duplicate extractions were 9.2% for phenobarbital and 15.4% for phenytoin. Carbamazepine and carbamazepine-10, 11-epoxide were determined by high performance liquid chromatography (Westenberg and de Zeeuw, 1976).

RESULTS

Phenobarbital. This drug was found to be distributed uniformly between the gray matter of the cerebral cortex and the underlying white matter (Table 1). The plasma levels ranged from 4.2μ g/ml to 38.3μ g/ml (mean, 14.4μ g/ml). The mean gray matter/plasma ratio was 0.74 ± 0.05 (SE). There was a significant correlation between plasma and brain levels for both gray matter ($r = 0.97$, $p < 0.001$) and the white matter ($r = 0.98$, $p < 0.001$). The white:

TABLE I

CONCENTRATIONS OF PHENOBARBITAL IN PLASMA, GRAY MATTER AND WHITE MATTER

PATIENT NO.	PLASMA (μg/ml)	GRAY MATTER (μg/gm)	WHITE MATTER (μg/gm)	GRAY/PLASMA RATIO	WHITE/GRAY RATIO
1	4.2	4.1	3.4	0.98	0.83
2	5.2	4.3	4.8	0.83	1.11
3	6.2	4.1	4.7	0.66	1.15
4	11.5	7.3	8.9	0.63	1.22
5	11.8	9.0	10.6	0.76	1.18
6	14.0	11.2	12.0	0.80	1.07
7	14.8	10.9	11.1	0.74	1.02
8	16.5	7.7	10.3	0.47	1.34
9	21.3	14.7	14.8	0.69	1.01
10	38.3	33.8	34.1	0.88	1.01
				0.74 ± 0.05*	1.10 ±0.04*

* Mean ± SE

TABLE II

CONCENTRATIONS OF PHENYTOIN IN PLASMA, GRAY MATTER AND WHITE MATTER

PATIENT NO.	PLASMA (μg/ml)	GRAY MATTER (μg/gm)	WHITE MATTER (μg/gm)	GRAY/PLASMA RATIO	WHITE/GRAY RATIO
11	4.2	5.1	7.3	1.21	1.44
12	-	17.0	23.0	-	1.35
13	7.1	12.2	16.0	1.72	1.31
14	11.0	19.1	29.9	1.74	1.57
15	17.0	18.6	24.4	1.09	1.31
				1.44 ± 0.17*	1.39 ±0.0 5*

*Mean ± SE

TABLE III

CONCENTRATIONS OF CARBAMAZEPINE* IN PLASMA, GRAY MATTER AND WHITE MATTER

PATIENT NO.	PLASMA (μg/ml)	GRAY MATTER (μg/gm)	WHITE MATTER (μg/gm)	WHITE/GRAY RATIO
16	6.9	4.5	6.0	1.34
17	—	1.4	1.5	1.07

*Carbamazepine-10,11-epoxide not detected

gray phenobarbital concentration ratios ranged from 0.83 to 1.34 with a mean of 1.10 ± 0.04 (SE).

Phenytoin: There was a considerable difference between the phenytoin concentrations in gray and white matter as shown in 5 patients in Table II. Plasma levels in 3 of the patients ranged from 7.1 μ g/ml to 17.0 μ g/ml, which is within the usual therapeutic range observed in patients receiving this drug at our Institute (7 μ g/ml to 20 μ g/ml). There was a good correlation between the drug concentrations in plasma and gray matter ($r = 0.86$), with a gray matter/plasma ratio of 1.44 ± 0.17 (SE) which is 1.9-fold greater than that of phenobarbital. Phenytoin concentrations averaged 14.2 μ g/gm in gray matter and 20.1 μ g/gm in white matter. Hence, white matter contained an average concentration of drug 1.4-fold higher than that of the gray matter of the cerebral cortex.

Carbamazepine. Table III shows the relative distribution of carbamazepine in gray and white matter obtained from two young adult females with temporal lobe epilepsy. Carbamazepine-10, 11-epoxide, a major metabolite of carbamazepine with anticonvulsant properties, was not detected in these samples (Morselli, 1975). However, this metabolite was not detected (less than 1 μ g/ml) in the plasma of one of the patients at the time of surgery.

DISCUSSION

Although there is no proof of the existence of specific binding of anticonvulsants by receptors in the brain, anticonvulsant activity does parallel brain concentration in the experimental animal (Baumel et al., 1973). An example involves the correlation between brain concentrations of phenytoin and phenobarbital and protection against maximal electroshock seizures in the rat (Leppik and Sherwin, 1977). As the plasma concentration:response curve represents a quantitative refinement of the dose:response curve, correlations involving concentrations of drugs in the brain, and especially in gray and white matter, offer additional refinements. The meaning of the plasma concentration in relation to drug in the brain is given additional perspective. Sufficient data are available in the literature (Houghton et al., 1975; Rapport et al., 1975; Vajda et al., 1974) and in Tables I and II with phenobarbital and phenytoin to reveal good correlations between plasma and brain concentrations. Effective plasma levels of phenobarbital (> 15 μ g/ml) are approximately 2-fold greater than those of phenytoin (> 10 μ g/ml) which may reflect the differences in their gray matter/plasma ratios. It is emphasized that the samples of cerebral cortex and subcortical white matter assayed in the present study were obtained from relatively normal portions of surgical specimens. The data presented illustrate differences in distribution between gray and white matter of some commonly used anticonvulsant drugs. Phenobarbital was found to be distributed equally between gray and white matter, while phenytoin concentrations were relatively higher in white matter. Phenobarbital is less lipophillic than the other anticonvulsants studied, and is only 46% bound by the plasma proteins at therapeutic concentrations (Maynert, 1972). These human data are in agreement with radioautographic studies of drug distribution in the cat and monkey after intravenous infusion of drugs (Roth and Barlow, 1961; Van der Kleijn et al., 1973).

The accumulation of phenytoin in white matter may reflect nonspecific binding to brain proteins and phospholipids, both of which are present in relatively higher concentration in white matter. Phenytoin is approximately 90% bound by plasma proteins at the usual therapeutic levels and its partition coefficient (1-chlorobutane/water) is 1.9 which is considerably higher than the value of 0.4 reported for phenobarbital (Bush and Sanders-Bush, 1972). Goldberg et al. (1976) have speculated that such binding may contribute to the action of phenytoin as a membrane stabilizer. Rapport et al. (1975) observed that small samples taken from sites of maximal focal epileptogenic activity, with histological evidence of gliosis, scarring and neuronal loss, contained significantly less phenytoin than did samples from normally appearing brain. They postulated that this could be due to reduced regional blood flow or a decreased number of binding sites.

Regional brain analysis of carbamazepine showed relatively higher concentrations in white than in gray matter. Similar concentrations of carbamazepine have been reported in human brain obtained at operation from patients with brain tumors (meningiomas), but gray and white matter were not separated (Morselli, 1975). A major metabolite, carbamazepine-10, 11-epoxide, the concentration of which may range from 5% to 55% (mean 21.4%) of the total plasma level (Eichelbaum et al., 1976), was not detected in brain, in this study. Carbamazepine is approximately 75% bound by plasma proteins. In contrast, the epoxide metabolite is only 50% protein bound (Morselli, 1975).

The data in this study revealed substantial differences in the relative distribution of anticonvulsant drugs between the cerebral cortex, the subcortical white matter, and plasma. These differences must be taken into account in evaluating pharmacological or neurophysiological studies involving the determination of the relative concentrations of these drugs in the brain.

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