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Alteration of ^{14}C-Dextroamphetamine Sulfate Absorption by Carbonic Anhydrase Inhibition* (33984)

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Pretreatment with acetazolamide has been shown to alter brain levels of certain drugs. Following acetazolamide treatment in rabbits Kelentey *et al.* (1) found the level of penicillin increased in brain and decreased in blood. They concluded that the increased brain levels were the result of increased permeability of the blood–brain barrier. Reed (2) found that acetazolamide treatment increased the duration of pentobarbital sleeptime in rats and the brain "pentobarbital space" was increased in treated animals during the first 20–40 min after drug administration.

Maren *et al.* (3) characterized acetazolamide as a pure carbonic anhydrase inhibitor; *i.e.*, it exerts no action either *in vivo* or *in vitro* other than the inhibition of carbonic anhydrase, the enzyme which reversibily catalyzes the interconversion of carbon dioxide and carbonic acid.

The present study was conducted to determine the effect of carbonic anhydrase inhibition on rat brain uptake of ^{14}C-dextroamphetamine sulfate, a drug producing its primary pharmacological effect in the central nervous system.

Materials and Methods. Male, Holtzman rats (140–185 g), fasted 6–12 hr, were treated with acetazolamide[1] (200 mg/kg, sc) 30 min prior to the administration of ^{14}C-*d*-amphetamine sulfate[1] (2 μCi/mg). Control animals were given an equivalent volume of saline in place of the acetazolamide. At various time intervals following the *d*-amphetamine injection, the animals were sacrificed by decapitation. Free-flowing blood was collected in tubes containing potassium oxalate. After centrifugation a 0.1-ml aliquot of plasma was taken for analysis. The brain was perfused by injecting 10 ml of saline into each carotid artery to minimize errors derived from the radioactivity in blood. Samples were taken from the frontal lobes of the cerebrum for

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1 Acetazolamide was a gift from Lederle Laboratories; ^{14}C-dextroamphetamine sulfate was a gift from Smith, Kline, and French Laboratories.

TABLE I. The Effect of Acetazolamide Treatment on Brain and Blood Levels of ^{14}C-Dextroamphetamine Administered Intraperitoneally.[a]

Time (min)	Brain levels (dpm/100 mg of tissue ± SE)		Plasma levels (dpm/0.1 ml of plasma ± SE)	
	Control	Acetazolamide	Control	Acetazolamide
5	1974 ± 254	3224 ± 240[b]	546 ± 50	898 ± 53[c]
10	3258 ± 382	4056 ± 320	571 ± 72	791 ± 5[b]
15	4112 ± 242	3840 ± 288	622 ± 64	670 ± 67

[a] Acetazolamide (200 mg/kg, sc) was administered 30 min prior to an intraperitoneal injection of ^{14}C-*d*-amphetamine (5 mg/kg; 2 μCi/mg). Control animals were given saline in place of acetazolamide. Each value represents the mean of 4 animals ± SE.

[b] $p < .05$.

[c] $p < .01$.

analysis of total radioactivity since it has been reported that almost all of the activity (>90%) is associated with the parent compound (4).

Both brain and plasma samples were prepared for liquid scintillation counting by the method of Bruno and Christian (5). After preparation the samples were refrigerated until counted in a Packard Tri-Carb scintillation spectrometer (model 3003). ^{14}C-Benzoic acid in toluene was employed as an internal standard in each sample.

Results and Discussion. In the first experiment *d*-amphetamine (5 mg/kg) was administered intraperitoneally and the animals were sacrificed at intervals of 5, 10, and 15 min. As shown in Table I, treatment with acetazolamide significantly increased the brain levels of the labeled drug at the 5-min interval. Differences observed at the 10-and 15-min intervals were not significant at the .05 level (Student's *t* test). Plasma levels of *d*-amphetamine were increased at all time intervals. It is apparent that plasma and brain levels are correlated, and that brain levels in acetazolamide-treated animals only reflect the higher plasma levels. This can be clearly seen by comparing brain-to-plasma ratios calculated on the basis of dpm/100 mg brain tissue:dpm/0.1 ml of plasma. No significant differences were found between the ratios derived from treated and control animals. However, the increased plasma levels in the treated animals do suggest that the absorption of *d*-amphetamine from the intraperitoneal injection site was more rapid.

When *d*-amphetamine (2.5 mg/kg) was injected intravenously and the animals were sacrificed at intervals of 2.5, 5, and 10 min, the brain and plasma levels of labeled drug in treated animals did not differ significantly from the corresponding levels in control animals at any of the time intervals (Table II). These results indicate that acetazolamide treatment did not increase the permeability of the blood–brain barrier to *d*-amphetamine. It should be noted, however, that in treated animals brain levels were slightly higher and plasma levels slightly lower than in control animals. This resulted in decreased brain-to-plasma ratios in the treated animals. These data are consistent with the concept that most drugs penetrate the central nervous system in the un-ionized form (6, 7). Mitchell and Ogden (8) have shown that the pH of rat blood decreases approximately 0.2 pH units following acetazolamide treatment (200 mg/kg). These results were also confirmed in our laboratory. Since *d*-amphetamine is a basic drug, an acidic shift in pH would decrease the proportion of the un-ionized form of the drug, and consequently, there would be a decrease in the brain level of the drug.

In an additional experiment *d*-amphetamine (5 mg/kg) was injected intraduodenally and the animals were sacrificed at intervals of 5, 10, and 15 min. Treatment with acetazolamide significantly increased both the

TABLE II. The Effect of Acetazolamide Treatment on Brain and Plasma Levels of ^{14}C-Dextroamphetamine Administered Intravenously.[a]

Time (min)	Brain levels (dpm/100 mg of tissue ± SE)		Plasma levels (dpm/0.1 ml of plasma ± SE)	
	Control	Acetazolamide	Control	Acetazolamide
2.5	3384 ± 192	3320 ± 146	462 ± 66	503 ± 32
5	3568 ± 8	3198 ± 196	393 ± 46	415 ± 16
10	3002 ± 58	2998 ± 188	299 ± 18	377 ± 28

[a] Acetazolamide (200 mg/kg, sc) was administered 30 min prior to an intravenous injection of [14]C-*d*-amphetamine (2.5 mg/kg; 2 μCi/mg). Control animals were given saline in place of acetazolamide. Each value represents the mean of 3 animals ± SE.

brain and plasma levels of labeled drug at the 5- and 10-min intervals (Fig. 1). Brain-to-plasma ratios were not significantly different at any of these intervals. The increased plasma levels, however, indicate an alteration in the absorption of the drug from the duodenal injection site.

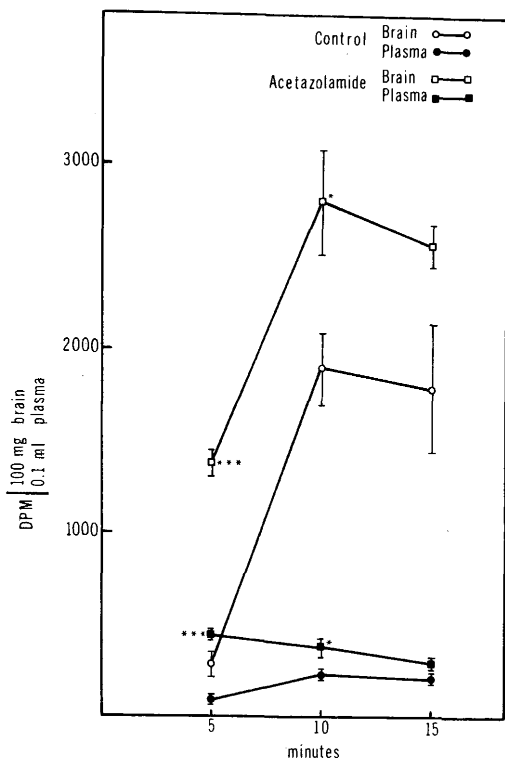

FIG. 1. Brain and plasma levels of ^{14}C-dextroamphetamine administered intraduodenally following acetazolamide pretreatment (200 mg/kg, sc); each point represents a mean of 4 animals; $*p<.05$; $**p<.001$.

These data are consistent with current concepts of drug absorption from the intestine (9, 10). In addition, Parsons (11) has found that the slightly acidic pH (6, 7) normally maintained in the upper intestine of the rat became more alkaline (pH 7.0) following acetazolamide treatment. Thus, an increase in pH in the intestinal milieu would result in an increase in the proportion of the unionized form of the basic *d*-amphetamine and, in turn, increase the rate of its absorption. Studies are in progress to investigate the mechanism involved in this altered absorption so the scope of this finding can be broadened to include all drugs.

Summary. Acetazolamide treatment does not increase the permeability of the blood–brain barrier to [14]C-*d*-amphetamine but does alter the absorption of this drug from intraperitoneal and intraduodenal injection sites. The increased rate of absorption of *d*-amphetamine from the duodenum is inferred to be the result of a pH change produced by the inhibition of carbonic anhydrase.

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Inhibition of the Binding of Cystine to Proteins by Glutamic Acid* (33985)

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Cystine in proteins may participate in peptide bonds, disulfide bonds, or both. The turnover of half-cystine bound to protein by disulfide bonds only is very much more rapid than is that bound by peptide bonds (1, 2). *In vivo,* this turnover of disulfide-bound half-cystine has been reported to be mediated by a disulfide–sulfhydryl interchange enzyme (3–5).

During the study of sulfur amino acid metabolism in regenerating wound tissue, we had shown that labeled peptide-bound half-cystine in the tissue protein of rats can be distinguished from that bound to the protein by disulfide bonds exclusively (1, 6). To verify these results *in vitro,* the uptake of cystine by proteins in fresh tissue homogenates was studied. Only a relatively small amount of labeled cystine became associated with the homogenate proteins by means of peptide bonds. In an attempt to increase peptide-bound ^{35}S-cystine incorporation, the homogenates were incubated with mixtures of amino acids—to no avail. Not only was there no increased incorporation of peptide-bound cystine, but the presence of the amino acids appeared to cause a diminution in the disulfide-bound half-cystine which became attached to the protein. This effect was soon traced to the presence of glutamic acid in the mixtures.

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A few characteristics of the inhibition of the sulfhydryl–disulfide interchange reaction by glutamic acid are presented below. Other parameters of the reaction are presently under investigation.

Experimental Methods. In early experiments, we compared the uptake of labeled cystine by native and denatured tissue proteins. When once it had been established that the effect of the glutamic acid is nonenzymatic *in vitro,* much of the subsequent work was carried out on protein from heat-denatured homogenates. Inactivation of tissue proteins is of obvious importance to prevent metabolic structural changes when studying the inhibitory capacity of glutamic acid or related compounds.

Rat tissue homogenates were prepared in an all-glass homogenizer with about 2–4 vol of 0.05 *M* tricine buffer at pH 7.3–7.5. After diluting the homogenate with 30–50 vol of buffer, it was heated in a water bath at 80–85° for 5 min and then filtered several times through glass wool. The filtrate was diluted with buffer to contain approximately 0.7–1.0 mg of protein/ml.

Experiments on the binding of labeled cystine were carried out at room temperature by mixing 2.0 ml of diluted tissue homogenate with 2.0 ml of glutamic acid solution, adjusted to the appropriate pH, and 2.0 ml of buffer containing 0.01 μCi of ^{35}S-L-cystine. The control was a similar mixture, containing 2.0 ml of tricine buffer instead of the gluta-