duplicate assays using both separation procedures and found that they yielded essentially identical results.

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Effects of Anthranil-, Benzo-, and Salicyl-Hydroxamic Acids on Mouse Brain Amines and L-DOPA Decarboxylase.* (31421)

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Anthranil-hydroxamic acid (AHA) lowers norepinephrine but not 5-hydroxytryptamine (5-HT) in rat brain(1). It was therefore of interest to determine the effect of benzohydroxamic acid (BHA) and salicyl-hydroxamic acid (SHA) on the concentration of these amines in brain. Mice were used in these experiments and the effects of the injection of the 3 hydroxamic acids were compared. Estimations of brain L-DOPA decarboxylase activity were also made.

Methods. AHA was obtained from Hynes Chemical Co., Durham, N. C.; BHA from Eastman Organic Chemical Co.; and SHA from Aldrich Chemical Co. Mice were injected intraperitoneally with 1 mg/g AHA and BHA, and 0.8 mg/g SHA in saline. Brains containing BHA or SHA were removed at intervals after injection and homogenized in 5 ml H_2O. Five ml 10% trichloracetic acid and 0.2 ml 10% $FeCl_3$ were then added. The mixture was filtered and the colored filtrate was read in the Coleman Spectrophotometer at 520 mμ. AHA was not extracted by H_2O so these brains were homogenized in 5 ml n-butanol and 2 ml acidic $FeCl_3$. This mixture was filtered and the colored butanol was read at the same wave length.

Mice were given hydroxamate intraperitoneally and then brain L-DOPA decarboxylase was measured manometrically(2). 0.28 g brain in M/15 phosphate buffer pH 7 was used with 3 μg pyridoxal phosphate and 4 mg DL-DOPA.

Two brains were pooled for each amine estimation. 5-HT assays are described elsewhere(1). Total catecholamines were measured in brain and cerebellum by the method of Potter *et al*(3) in mice receiving BHA and SHA. In this method brains are homogenized

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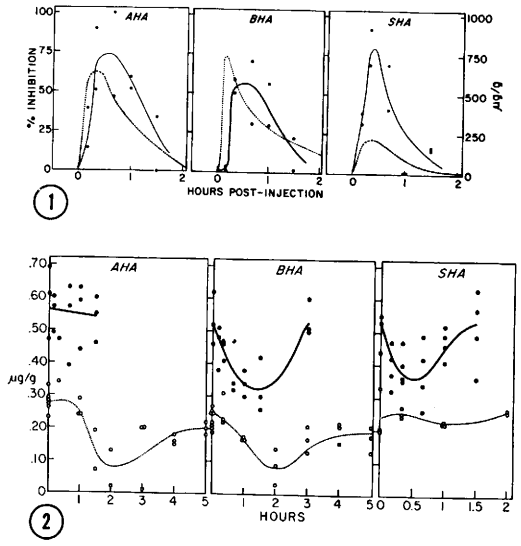

FIG. 1. Percentage of brain L-DOPA decarboxylase inhibition and brain concentration of hydroxamate after injection.

FIG. 2. Brain amine levels after hydroxamate injection.

in n-butanol. Since AHA was extracted with the catecholamines and appeared in the final solutions, brains containing AHA were homogenized in HCl which was then extracted into butanol as in the method of Shore and Olin(4). Catecholamines were then extracted from butanol into 10-11 ml 0.1 HCl. 2 ml was adjusted to pH 6.0 with 1 ml 0.3 N $NaHCO_3$ and 1 ml acetate buffer. 0.1 ml 0.25% $K_3Fe(CN)_6$ was added and 3 minutes later 1 ml of 2% ascorbic acid mixed 1:9 with 20% NaOH was added. Glass-distilled H_2O was added to a volume of 10 ml. Faded blanks were made by adding ascorbate 4 minutes after NaOH. A standard curve was made with norepinephrine.

Results. Fig. 1 shows the percentage of brain L-DOPA decarboxylase inhibition and the brain concentration of hydroxamate after injection. Each point on the figure refers to the left ordinate and represent a sample from one mouse. The solid line represents the average of these points. The broken line refers to the right ordinate and represents the average concentration of hydroxamate in 3 different brains at 10, 20, 40, 60 and 120 minutes. All 3 hydroxamates enter the brain rapidly, although SHA shows the lowest concentration in the brain. Inhibition begins shortly after the peak hydroxamate concentration and disappears in about the same time. Fig. 2 shows brain amine levels after hydroxamate injection. Each solid dot represents 5-HT in 2 pooled brains and the average is represented by the solid line. Each circle represents total catecholamines in 2 pooled brains and the average is shown by the broken line. There is a common ordinate scale. The changes in 5-HT do not outlast the presence of hydroxamate in the brain or the decarboxylase inhibition, while changes in catecholamine levels are more enduring. The effects of AHA are similar to those found in the rat but not so pronounced.

Discussion. While it might be anticipated from the fact that all 3 hydroxamates inhibit L-DOPA decarboxylase they would also all lower both 5-HT and catecholamines, this is realized only with BHA. The failure of AHA to lower 5-HT and that of SHA to lower catecholamines can only be explained on the basis of selective depletion of these amines or selective action at certain other sites of amine formation. It is of interest that 3 compounds so closely related should have selective effects.

Summary. Anthranil-, benzo- and salicyl-hydroxamic acids were given intraperitoneally to mice. Brain L-DOPA decarboxylase activity was reduced shortly after the hydroxamates entered the brain and returned to normal as the hydroxamates were eliminated. Salicyl-hydroxamic acid was the strongest inhibitor. Only benzo-hydroxamic acid lowered both 5-hydroxytryptamine and catecholamine levels in brain. Anthranil-hydroxamic acid lowered only catecholamine levels and salicyl-hydroxamic acid lowered only 5-hydroxytryptamine levels.

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