Effect of D-Amphetamine on Rat Brain Noradrenaline and Serotonin. (26540)

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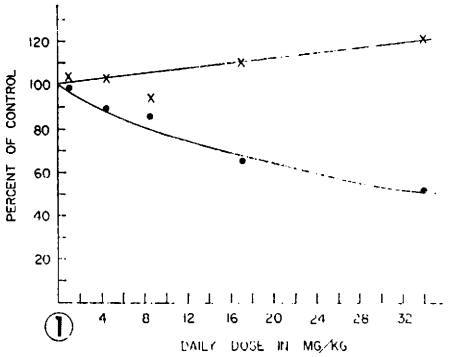
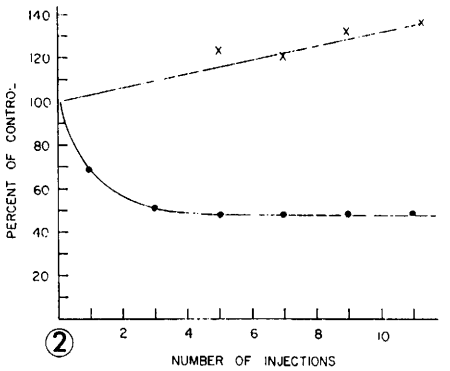
Recent interest in the role of noradrenaline and serotonin as possible neurohumors has stimulated investigation of the influence of various treatments on the biochemistry of these metabolites. Experimental conditions having differential effects on tissue levels of noradrenaline and serotonin may be of particular value in understanding the possible functions of these compounds in brain metabolism. The sedative reserpine, for example, depresses brain levels of both amines; however, further observations suggest that the effects of Rauwolfia alkaloids may be related to the changes in serotonin rather than noradrenaline. In rats and rabbits treated with a reserpine analogue, or cold stressed and treated with reserpine, noradrenaline levels were markedly depressed with a much smaller change in serotonin, and sedation was not produced(1). Iproniazid and other monoamine oxidase inhibitors elevated both noradrenaline and serotonin in rat and rabbit brain and caused central excitation, whereas in cats and dogs only serotonin was markedly elevated and pronounced central excitation was not produced (2). Vogt reported that in several species, drugs causing hypothalamic stimulation lowered hypothalamic noradrenaline levels(3). During a study of the effects of a number of drugs on rat brain metabolism it was noted that repeated injections of amphetamine or ephedrine lowered noradrenaline levels. The results of a further study with D-amphetamine are given in this report.

Materials and methods. Drugs were given twice daily at 8 a.m. and 4 p.m. by subcutaneous injection to groups of 5-10 male Holtzmann rats weighing 170-200 g. The animals were killed by decapitation, the whole brain removed and immediately frozen on dry ice. Except where otherwise noted the rats were killed 1 hour after last injection. The tissue was extracted as described by Shore and Olin(4), and noradrenaline determined fluorometrically by the method of Von Euler and Floding(5) using an Aminco-Bowman spectrophotofluorometer, with activation at 390 mμ and fluorescence measurement at 490 mμ. Serotonin was also determined in the same acid extract by the fluorometric method of Bogdanski *et al.*(6). For noradrenaline, the average value obtained with 8 separate groups of control animals was .32(.19-.50) γ per g wet weight. For serotonin, the average value obtained was .58(.45-.72) γ per g wet weight. The variation in values obtained with samples of the same homogenate extracted and assayed together was $\pm$ 8%. Where several treatments were compared the tissues were extracted and assayed together. Monoamine oxidase was determined by Warburg assay using tyramine as substrate.

Results. Progressive doubling of the daily doses of D-amphetamine caused progressively greater decreases in brain noradrenaline levels and a slight increase in serotonin levels (Fig. 1). Fig. 2 shows the effects of from one to 11 injections of 17 mg/kg of D-amphetamine. Noradrenaline levels reached a minimum after 5 injections, serotonin levels continued to rise slightly with repeated injections. Lowering of brain noradrenaline and elevation of serotonin persisted for at least 120 hours after last injection of D-amphetamine (Fig. 3). Table I shows the effect of combined treatment with iproniazid and amphetamine. There was no detectable monoamine oxidase activity in the brains of animals treated with iproniazid, alone or with amphetamine. Iproniazid alone gave the expected increase in both noradrenaline and serotonin. D-Amphetamine antagonized the elevation of noradrenaline by iproniazid, and moderately enhanced the elevation of serotonin.

Discussion. It does not seem probable that the decrease in noradrenaline following D-amphetamine administration is due to any change in an exchange across the blood brain





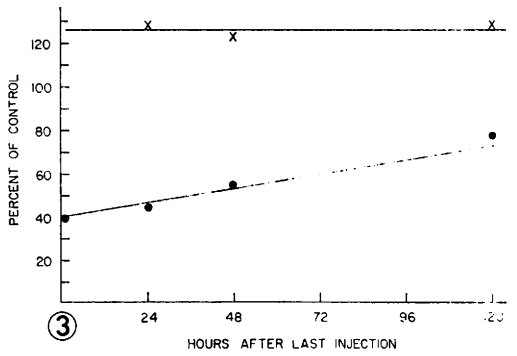


FIG. 1. Brain noradrenaline ●—●, and serotonin ×—×, in rats given D-amphetamine sulfate, 2 subcut. injections a day for 2 days and 1 injection on the third day.

FIG. 2. Brain noradrenaline ●—●, and serotonin ×—×, in rats given twice daily subcut. injections of 17 mg/kg of D-amphetamine sulfate.

FIG. 3. Brain noradrenaline ●—●, and serotonin ×—×, in rats given D-amphetamine sulfate, 2 subcut. injections a day for 2 days, 1 injection on the third day, then killed at intervals thereafter.

barrier. Although there is no direct evidence on losses of brain noradrenaline to the blood stream, injected noradrenaline is not taken up from the blood at an appreciable rate(7). It has been suggested that monoamine oxidase is

TABLE I. Brain Noradrenaline and Serotonin Levels in Rats Given Twice Daily Injections of Iproniazid Alone or with D-amphetamine for 3 Days.

Drug	Dose in mg/kg/day	Brain level as % of control: Nor-adrenaline	Brain level as % of control: Sero-tonin
Iproniazid	20	179	230
D-amphetamine sulfate + Iproniazid	34 + 20	100	280
D-amphetamine sulfate + Iproniazid	17 + 20	135	290

a major pathway for degradation of noradrenaline in brain(8). The level of noradrenaline in animals treated with iproniazid plus amphetamine is lower than the level in animals given iproniazid alone (Table I). Since monoamine oxidase is completely inhibited in these animals it does not appear that the effect of amphetamine in lowering noradrenaline levels is due to any effect on monoamine oxidase.

A further possibility is that the decrease in brain noradrenaline following treatment with amphetamine could be due to a decrease in synthesis. It has been suggested that amphetamine mimics the action of noradrenaline centrally(9). If this is true one might expect prolonged treatment with amphetamine to activate a feed back mechanism depressing the synthesis of noradrenaline. In any case, the differential effect of D-amphetamine on noradrenaline and serotonin may serve as another illustration of the existence of independent metabolic pathways for the 2 postulated neurohormones.

Summary. The treatment of rats with D-amphetamine sulfate lowered brain noradrenaline and slightly elevated brain serotonin. These effects lasted for several days after discontinuing treatment. D-amphetamine sulfate antagonized the elevation of brain noradrenaline levels by iproniazid.

1. Brodie, B. B., Finger, K. F., Orlans, F. B., Quinn, G. P., Sulser, F., *J. Pharm. Exp. Therap.*, 1960, v129, 250.

2. Spector, S., Shore, P. A., Brodie, B. B., *ibid.*, 1960, v128, 15.

3. Vogt, M., *J. Physiol.*, 1954, v123, 451.
4. Shore, P. A., Olen, J. S., *J. Pharm. Exp. Therap.*, 1958, v122, 295.
5. Von Euler, U. S., Floding, I., *Acta Physiol. Scand.*, 1955, Suppl. 33, v118, 45.
6. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharm. Exp. Therap.*, 1956, v117, 82.
7. Schayer, R. W., *J. Biol. Chem.*, 1951, v189, 301.
8. Grout, J. R., Creveling, C. R., Calon, D., *Fed. Proc.*, 1960, v19, 297.
9. Brodie, B. B., Shore, P. A., *Ann. N. Y. Acad. Sci.*, 1957, v66, 631.

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Biological Assay of Proteolytic Enzymes Capable of Debriding Third Degree Burn Eschars. (26541)

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In attempting to identify the proteolytic enzyme or enzymes in *Clostridium histolyticum* culture filtrates which were responsible for the ability of these preparations to debride third degree burn eschars, it was found that adequate methods of bioassay were lacking. Although in recent years a variety of enzymes have been proposed as agents for removal of burn slough, methods for comparing the biological potency of these enzymes have not been available. Mention has been made of the use of laboratory animals such as the guinea pig(1,2), dog(3,4) or rabbit (5,6) but no attempt has been made to develop a quantitative procedure.

The present paper describes a method for biological assay of proteolytic enzymes capable of debriding third degree burn eschars. A suitable bandaging technic for maintaining the enzyme solution in contact with the burned surface was developed and a scale for assessing the effectiveness of debridement was chosen. In addition, 2 examples of the use of this assay are given; 1) evaluation of debriding activities of various enzyme preparations, and 2) determination of the effect on debriding activity of pre-treatment of the burn eschar with pHisoHex and with petrolatum.

Materials and methods. Four proteolytic enzymes were used. Two of the enzyme preparations, designated as 0-22% enzymes and 30-35% enzymes, were obtained from *C. histolyticum*, H-4, filtrates by a modification of the procedure developed by deBellis *et al.* (7). The mold proteinase from culture X-108 (Lot No. 7-1162D-12) was kindly supplied by Frank B. Ablondi, Biochemical Research Section, Research Division, American Cyanamid Co. Purified crystalline Trypsin (approximately 50% $MgSO_4$, Lot TP11) was purchased from Worthington Biochemical Laboratory. All enzyme preparations were dissolved in 0.1 M tris (hydroxymethyl) aminomethane buffer, pH 7.5, containing 0.01 M $CaCl_2$ and 0.5% proteose peptone (Difco), except the mold enzyme which was dissolved in 5% proteose peptone, pH 7.5. In addition, 1000 units of potassium penicillin G and 500 μg of streptomycin were added for each ml of test solution to inhibit bacterial contamination.

Hartley strain guinea pigs (350-800 g) were anesthetized with sodium pentobarbital and the entire dorsal area was clipped as closely as possible. Four full thickness third degree burns with a diameter of approxi-

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