Reports

On the Physiologic Significance of Monoamine Oxidase in Brain

The discovery of norepinephrine in brain is creating considerable interest concerning the role of this neurohumoral agent in the central nervous system (*1*). Its precise function is still unknown, but the similar patterns of distribution of bound norepinephrine and serotonin, particularly the high levels in the hypothalamus, permit speculation that both amines act in central regulatory mechanisms.

If norepinephrine in brain acts as a chemical transmitter, there must be a mechanism to prevent accumulation of the free substance at receptor sites. The mode of inactivation of norepinephrine released from storage in brain becomes therefore of obvious importance. Several enzymes can metabolize norepinephrine and epinephrine *in vitro* (*2*), but the extent to which each participates *in vivo* is controversial. Monoamine oxidase has been considered in many reports, but a number of objections have been raised against the view that this enzyme is functionally significant in destroying norepinephrine that is released from adrenergic nerves. Perhaps the most important objection is the relatively slow action of this enzyme on catechol amines *in vitro*; in fact, its more rapid action on serotonin has suggested to some workers that this indole may be the only important physiological substrate (*3*). But we may well ask whether the properties of the enzyme in a homogenate can be translated into a clear picture of its action *in vivo*. If brain monoamine oxidase is concentrated at adrenergic nerve endings, as is reported for the enzyme in sweat glands of the horse (*4*), it could almost instantly destroy minute amounts of hormone that are liberated at receptor sites, regardless of the relatively low activity of the enzyme after dilution in tissue homogenates.

Conclusions concerning the physiologic importance of monoamine oxidase are also questionable when they are based on studies of the metabolism of injected norepinephrine. The fate of norepinephrine, when administered intravenously, could be quite different from its fate when it is released from nerves in close proximity to a high concentration of monoamine oxidase. Thus injected norepinephrine might contact enzyme systems not present in adrenergic nerves and yield products not representative of the metabolic fate of amine released from nerves.

The present report describes experiments which implicate monoamine oxidase as the enzyme mainly responsible for the physiologic inactivation of both serotonin and norepinephrine in brain. In these experiments the amines were measured by specific fluorometric procedures which assay total (bound plus free) amines (*5*). Their degradation was assumed to be catalyzed by monoamine oxidase if blocked by known monoamine oxidase inhibitors.

The rates of metabolism of the amines were measured in homogenates of rabbit brain stem prepared in 6.5 volumes of 0.1*M* phosphate buffer at *p*H 7.4. Norepinephrine or serotonin was added to yield a concentration of 10 μg/ml, and the preparations were incubated in air at 37°C. Under these conditions, about half the serotonin disappeared in 10 minutes as compared with 50 minutes for norepinephrine (*6*). The metabolism of the amines was almost completely suppressed by the monoamine oxidase inhibitors, iproniazid (10^{-3} to $10^{-4}M$) and ephedrine ($10^{-2}M$). These results led to the conclusion that both amines were destroyed in the homogenates by the action of monoamine oxidase only.

The importance of brain monoamine oxidase *in vivo* was demonstrated by the substantial rise in levels of the amines in brain stem after administration of iproniazid. When iproniazid was given subcutaneously to four rabbits in doses of 50 mg/kg for 4 days, the levels of serotonin in brain stem increased from about 0.7 to 1.5 μg/g and those of norepinephrine from about 0.5 to 1.5 μg/g.

Destruction of the amines at their actual sites of release in brain was studied through the action of reserpine in freeing both norepinephrine and serotonin from their storage depots (*7*). After administration of reserpine to rabbits, brain levels of the amines declined as they were released and enzymatically destroyed (Fig. 1). However, pretreatment of the animals with iproniazid completely blocked the metabolism of the released amines (Table 1), suggesting that they were acted on by monoamine oxidase only. It could be argued that iproniazid prevented the release of the amines by reserpine, but earlier studies have indicated that iproniazid does not affect the liberation of serotonin from brain (*8*) or platelets (*9*).

It is noteworthy, however, that after administration of reserpine, norepinephrine and serotonin disappeared from brain at identical rates (Fig. 1), in marked contrast to the dissimilar rates in brain homogenates. This suggests that the rates of metabolism of the amines, after administration of reserpine, were limited not by the action of monoamine oxidase, but rather by the time required for the substances to be released and to contact the enzyme. This would be in accord with an earlier finding that considerable time is required for reserpine to free all the serotonin from platelets *in vitro* (*10*). It would thus follow that the actual rates of destruction of the amines at sites of their release in brain would be rapid in order to cancel out the preference of monoamine oxidase for serotonin as observed *in vitro*. This would hold true if the concentration of monoamine oxidase were very high at nerve endings.

The data presented here show that iproniazid, a potent inhibitor of monoamine oxidase (*11*), blocks the metabolism of brain norepinephrine and serotonin *in vitro* and *in vivo*, suggesting that monoamine oxidase in brain has a major role in the physiologic inactivation of

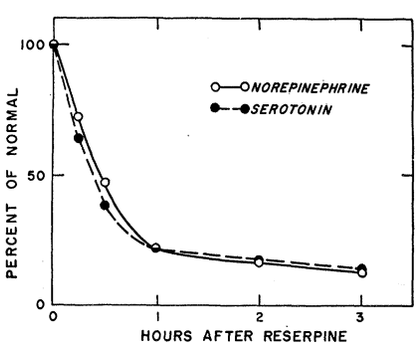

Fig. 1. Comparative rates of disappearance of norepinephrine and serotonin in rabbit brain stem after release by intravenous administration of reserpine (5 mg/kg). Points at zero time denote amine concentration in controls (norepinephrine, 0.5 μg/g; serotonin, 0.7 μg/g). Each value represents the average of from 2 to 4 animals.

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Table 1. Effect of iproniazid on metabolism of brain serotonin and norepinephrine after release by reserpine. The rabbits received 100 mg/kg of iproniazid intravenously. After 6 hours some animals received 5 mg/kg of reserpine intravenously. One hour later the animals were killed and their brain stems were analyzed. Values for amines in animals given iproniazid are somewhat higher than normal because the inhibition of monoamine oxidase causes levels to rise.

Injection	Serotonin level (μg/g)	Norepinephrine level (μg/g)
Iproniazid	1.00, 1.46	0.69, 0.61
Iproniazid + Reserpine	1.15, 1.08	0.64, 0.67, 0.57

both amines. Since iproniazid blocks the deamination of brain norepinephrine without disclosing another metabolic pathway, it seems unlikely that appreciable amounts of the "hallucinogenic" adrenochrome type of compound are formed in normal brain.

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Toxicologic Evaluation of Gibberellic Acid

Gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*, produces a diversity of responses in plants, perhaps the most striking of which is rapid elongation of shoots or acceleration of the rate of organ growth. Horticulturists and agronomists are currently making application of this agent to many economic and ornamental plants with the expectation of usefully modifying the normal growth habit (*1*). The agent is produced in practical quantities from filtrates of deep cultures of the fungus (*2*). Phinney (*3*) has reported that "Gibberellin-like" materials are present also in extracts of flowering plants, thereby indicating that these agents are natural constituents.

Plants are treated with the potassium salt of gibberellic acid (*4*), by spraying (concentrations of 1 μg to 1.0 mg/ml), by painting with a paste (0.5 to 1.0 percent), or by dipping seeds in solutions of 1.0 percent, or lower, concentrations. Thus concentrations of the agent which may be accidentally inhaled or ingested, or which may come into contact with the eye or skin, vary greatly. The residue remaining on treated plants at the time of harvesting appears to be negligible. Presently available analytical methods (sensitive to less than 1 μg/ml) are unable to detect the agent in harvested plants. Toxicologic studies of gibberellic acid, designed to expose any inherent toxicity for the human being or animal, are presented in this report.

Gibberellic acid was prepared either as a 30.0 percent aqueous solution by converting the acid to the sodium salt with sodium hydroxide or as a 50.0 percent concentration in carboxymethylcellulose suspension. For determinations of the acute intravenous toxicity, the appropriate concentrations were administered into the tail veins of Carworth female white mice in volumes of 0.5 ml or less at the rate of 1.0 ml/min. For determinations of acute oral toxicity, the appropriate concentrations were administered by stomach tube. The mice were observed frequently for several hours and then were held for 7 days, when some of the surviving mice were sacrificed; various tissues (*5*) were then examined grossly and prepared for histomorphologic studies.

Studies of the acute intravenous toxicity of gibberellic acid gave an LD_0 of 4.2 g/kg, and LD_{50} of 6.3 g/kg, and an LD_{100} of 8.7 g/kg. The signs of toxicity were nonspecific. No deaths and only minimal signs of toxicity were observed after the oral administration of 25.0 g/kg. Gross and histomorphologic studies did not reveal lesions or tissue changes that could be attributed to an effect of administration of gibberellic acid.

Twenty-seven male and 27 female Holtzman white rats were fed a diet containing 5.0 percent gibberellic acid. One-third of these animals were sacrificed after 5 weeks' feeding on the diet, and one-third after 8 weeks' feeding. The remaining rats are being continued on the diet. Three groups of control rats were fed the basal diet, and one group was sacrificed with each experimental group.

Body weights, food consumption, and hematologic values were normal for all groups of rats. Gross and histomorphologic studies of the various tissues (*5*) did not reveal lesions or alterations that could be attributed to the administration of gibberellic acid. Weights of organs were within normal limits.

Two groups of male and female Holtzman rats were exposed to an aerosol produced by spraying a solution containing 200 or 400 parts per million of gibberellic acid. The aerosol was produced continually for 10 minutes in a closed 88-1 chamber containing one group of rats. The rats then were held in the chamber for an additional 50 minutes. This procedure was repeated twice a day for 3 weeks. One-half of the rats were sacrificed at the termination of the study, one-fourth of the original group were autopsied after 1 month, and the remaining rats 2 months after the exposure. Control rats were exposed to an aerosol of the vehicle and sacrificed in the same temporal sequence. Gross and microscopic examinations of the various tissues (*5*) did not reveal abnormalities.

A single application of a 1.0 percent aqueous suspension of gibberellic acid to the eye of the rabbit did not produce immediate or delayed signs of irritation.

A concentration of 100 μg/ml in cultures of Rhesus testicular cells, Hela cells, or stable human amnion cells did not result in toxic reactions or in stimulation of cell growth. Repeated subcultures through several passages in the presence of the agent did not reveal cytotoxic activity. The yield and morphology of the cells were not influenced.

The order of acute and subacute toxicity of gibberellic acid is such that it is relatively harmless when administered orally, parenterally, by inhalation, or by topical application. This is the more remarkable since the agent is so potent that it may be employed effectively in amounts that leave no detectable residue on plants. The present evidence indicates that the agent presents no apparent hazard either to the individual who uses the material for agricultural purposes or to the individual who consumes products on which gibberellic acid or salts have been used.

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