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SHORT COMMUNICATIONS

The influence of epinephrine and norepinephrine on the accumulation of amphetamine-1-^{14}C by rat brain*

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IN THE course of recent experiments in this laboratory on the distribution of amphetamine in brain,[1] it was found that this substance was rapidly accumulated by brain and rapidly removed. Amphetamine therefore appeared to be a good indicator for studies on the effect of various treatments on the process of blood-brain transport. This report describes the positive effect of large doses of epinephrine and norepinephrine on the accumulation of amphetamine by brain.

A solution consisting of dl-amphetamine-1-^{14}C (0·3–0·7 μC/ml) and unlabeled dl-amphetamine to give a final concentration of free base of 0·9 μmole/ml was prepared in 0·9 per cent saline.† Other compounds tested were dissolved in this solution, all at a concentration of 0·75 μmole/ml. These large concentrations were employed since catecholamines are so rapidly metabolized.[2, 3] Fasted Wistar male rats (150–250 g) were injected intraperitoneally with 1 ml of the appropriate solutions per 100 g of body weight. At $\frac{1}{2}$ hr after injection, when the maximal amount of ^{14}C appeared in brain following the injection of amphetamine alone, the animals were decapitated and blood was collected in glassware dusted with anticoagulant. Brain tissue anterior to the first spinal nerve was removed, washed in saline, blotted and weighed. Ten per cent homogenates were prepared in 0·1 N HCl containing 4μg of amphetamine/ml to act as carrier. Brain-^{14}C was recovered and identified as amphetamine in the following manner. Protein was precipitated with trichloroacetic acid, and the supernatant solution obtained was extracted with benzene at pH 12–13.[4] The amine was recovered from the benzene by extraction with 0·1 N HCl. After freeze-drying, the residue was spotted on Whatman No. 1 paper and developed (descending technique) in *n*-butanol : acetic acid : water (50 : 40 : 10). Radioactivity of dried samples was combusted to CO_2 and measured as ion current with a vibrating reed electrometer (1 μC = 4·62 $\times$ 10^{-12} amperes).

The main findings are shown in Table 1. Both epinephrine and norepinephrine, but not normetanephrine, the major metabolite of norepinephrine,[2] definitely increased the concentration of amphetamine in brain. These are noteworthy effects in view of the difficulty in modifying the transport of compounds into brain.[5] The good recoveries of ^{14}C, which partitioned like amphetamine according to established procedures,[4] and the detection of single peaks of radioactivity corresponding to authentic amphetamine, make it very probable that the ^{14}C accumulated was amphetamine. Plasma radioactivity from a few animals was determined in order to evaluate the contribution of blood-^{14}C to the brain values. Assuming a 2·4 per cent contamination,[6] less than 1 per cent of the total brain-^{14}C could be attributed to blood. Direct determination of blood in brain[7] gave the following values: amphetamine-treated, 0·8 per cent; amphetamine + epinephrine, 0·8 per cent; amphetamine + norepinephrine, 0·9 per cent. The data on radioactivity of plasma, to date, however, do not permit an evaluation of the role of circulating ^{14}C in the production of increased content of amphetamine in brain by catecholamines.

The animals injected with amphetamine + epinephrine, or with amphetamine + norepinephrine, were unresponsive to external stimuli and somewhat ataxic, these effects being more pronounced in the animals treated with norepinephrine. This behavior contrasts with the hyperexcitability of animals injected with the same dose of amphetamine or amphetamine + normetanephrine. Control animals injected with epinephrine or norepinephrine alone were quiescent relative to animals injected with saline, but they were more alert than those given both amphetamine and catecholamine. This diminished alertness in the latter animals, however, could not have been due to the increase of

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† The amphetamines were kindly supplied by Smith, Kline and French Laboratories.

amphetamine in brain, since animals injected with amphetamine in doses twice those previously used, i.e., 1·8 μmoles/ml, had higher levels of amphetamine in brain than did those described in the table, and exhibited typical excitement. The possibilities that amphetamine promoted an increase in the concentrations of catecholamines in the brain, or that increased brain catecholamines resulted directly from the large doses employed, were not investigated.

Despite the lack of accumulation of epinephrine in brain during intravenous infusion, except to a very limited degree in the hypothalamus,[8] it is possible that the observed behavior of the rats is due to elevated cerebral catecholamines. This possibility must be considered for the following reasons:

(1) it has been reported that when epinephrine or norepinephrine is injected into the lateral ventricle anesthetic-like effects are produced;[9]

(2) it may be that the behavior change is mediated by changed hypothalamic concentrations of the catecholamines;[8]

(3) since amphetamine has a profound influence on the metabolism of the catecholamines,[3] general or local changes in the brain concentration of either epinephrine or norepinephrine, or both, may occur when amphetamine is administered together with the catecholamines.

TABLE 1. INFLUENCE OF EPINEPHRINE AND NOREPINEPHRINE ON THE ACCUMULATION OF AMPHETAMINE-1-^{14}C BY RAT BRAIN

Substance administered	Accumulation of ^{14}C by rat brain*	Percent recovery brain-^{14}C as amphetamine	R_f brain-^{14}C†
Amphetamine	1·07 ± 0·03 (8)	94	0·70–0·81 max = 0·72–0·74
Amphetamine + norepinephrine	1·49 ± 0·07 (8) $P < 0·005$	90	0·71–0·80 max = 0·73–0·76
Amphetamine + epinephrine	1·57 ± 0·09 (3) $P < 0·05$	90	0·69–0·80 max = 0·73–0·76
Amphetamine + normetanephrine	0·82, 0·86	—	—

* $\left(\frac{\text{Total brain-}^{14}\text{C/g wet wt}}{^{14}\text{C administered/kg body wt}} \times 10^3\right)$; mean values from number of animals in parentheses ± S.E.; P-values for comparisons with amphetamine alone.

† R_f of authentic amphetamine-^{14}C, 0·66–0·81; max = 0·76.

The effects shown suggest that epinephrine and norepinephrine, or perhaps metabolic intermediates, might regulate the uptake of at least some compounds by brain. That these effects might be more general is implied by the findings of others,[10] and is being investigated further with other classes of compounds. In the event that similar findings result from the continuous infusion of physiological quantities of catecholamines or by other interventions that may maintain a relatively high level of these compounds in blood, the data suggest the interesting possibility that the degree of action of amphetamine (and possibly of other neuro-active agents) might be regulated by circulating catecholamines.

Biochemical Research Laboratories
The Institute of Living
Hartford, Conn.

R. L. YOUNG*
M. W. GORDON

* Special Trainee of the National Institute of Neurological Diseases and Blindness (BT-589). Present address: Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri.

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Inhibition of oxidative phosphorylation by an antipyretic drug

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IN THE course of a study on the mechanism of the pharmacological action of antipyretics, an inhibitory effect "in vivo" on rat oxygen uptake and "in vitro" on isolated rat liver mitochondria was observed using 2-allyl-oxybenzamide, a salicylamide (2-hydroxybenzamide) derivative.[1]

The main results, obtained with rat liver mitochondrial preparations, are summarized in this report. Table 1 shows the effect of 2-allyl-oxybenzamide (AOB)* on the oxidative phosphorylation

TABLE 1. OXIDATIVE PHOSPHORYLATION IN PRESENCE AND IN ABSENCE OF 2-ALLYLOXYBENZAMIDE (AOB)

	substrate	AOB	O_2 uptake (μatoms/mg N)	P esterified (μmoles/mg N)	P/O
(a)					
	α-ketoglutarate	—	12·59	31·44	2·50
	α-ketoglutarate	0·002 M	3·90	10·51	2·70
	β-hydroxybutyrate	—	4·70	13·20	2·80
	β-hydroxybutyrate	0·002 M	1·67	4·30	2·57
	succinate	—	16·30	29·80	1·83
	succinate	0·002 M	16·50	23·60	1·43
(b)					
	DPNH	—	10·10	10·67	1·06
	DPNH	0·002 M	9·10	8·07	0·89
(c)					
	ferrocytochrome C	—	5·76	2·70	0·47
	ferrocytochrome C	0 002 M	6·10	2·74	0·45

The oxygen uptake was measured manometrically at 26 °C in the Warburg apparatus. The vessels contained 0·005 M $MgSO_4$, 0·03 M glucose, 0·0014 M ATP, 0·8 mg yeast hexokinase (Sigma, type II), 0·00001 M cytochrome C, 0·09 M sucrose, and:

(a) α-ketoglutarate (0·01 M), succinate (0·01 M) and β-hydroxybutyrate (0·02 M) as substrates, 0·03 M potassium phosphate buffer, pH 7.4, 0·00036 M $MnCl_2$, mitochondria (isolated in 0·25 M sucrose) 1 mg of N; time of incubation 20 min.;

(b) DPNH (0·002 M) as substrate, 0·009 M potassium phosphate buffer, pH 7·4, 0·04 M *tris* buffer, pH 7·4, 0·001 M ethylendiaminotetracetate, mitochondria (isolated in 0·25 M sucrose and pretreated for 15 min. at 0 °C with 0·075 M sucrose) 0·5 mg of N; time of incubation 15 min.;

(c) reduced cytochrome C as substrate, 0·009 M potassium phosphate buffer, pH 7·4, 0·02 M *tris* buffer, pH 7·4, 0·01 M ascorbate, 0·00036 M $MnCl_2$, 0·01 M KF, mitochondria (isolated in 0·25 M sucrose and pretreated for 15 min. at 0 °C with 0·075 M sucrose) 1 mg of N; time of incubation 30 min.;

* 2-allyl-oxybenzamide was kindly supplied by Cassella-Curta (Frankfurt/M).