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Effect of amphetamines on tryptophan concentrations in mice and rats

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(±)-Amphetamine and some of its analogues were administered intraperitoneally to mice and rats and the concentrations of tryptophan in tissues were analysed by fluorimetric and microbiologic techniques. The concentration of tryptophan in brain was markedly increased by (±)-amphetamine and reached a maximum about 80 min after drug administration. The effect was dose-dependent with a threshold dose below 1 mg/kg. (+)-Amphetamine was significantly more potent than *p*-chloroamphetamine and *p*-hydroxyamphetamine, indicating that the changes in tryptophan concentrations might be related to the central stimulating effect of the drugs. (±)-Amphetamine delayed the disappearance from brain of intravenously administered [^{3}H]tryptophan. Inhibition of monoamine oxidase by nialamide (100 mg/kg) and tryptophan hydroxylase by H 22/54 (500 mg/kg) had no effect on the tryptophan concentration in brain.

In recent years attention has been paid to the possible role of tryptophan metabolism in psychiatric disorders (Jepson & Spiro, 1960; Herzov & Rodnight, 1960; Greer, 1965; Allan & Holt, 1965; Dewhurst, 1968; Curzon & Bridges, 1970). Some psychotropic drugs have been shown to influence the concentration of tryptophan in tissues (Schubert, Fyrö & others, 1970; Tonge & Leonard, 1970; Leonard & Liska, 1970; Leonard & Shallice, 1971); treatment with (±)-amphetamine elevated the content of tryptophan in the mouse brain (Schubert & others, 1970). Since the brain concentration of tryptophan could be increased up to threefold by (±)-amphetamine treatment, a closer analysis of the observation has been made.

In this report different methods of measuring tryptophan have been used to examine the effect of (±)-amphetamine and some of its analogues on the tryptophan concentration in brain and other tissues. The effects of time- and dose of (±)-amphetamine on tryptophan concentrations in brain are described. To elucidate the mechanism of effect of amphetamine, the fate of intravenously administered [^{3}H]tryptophan has been determined.

METHODS

The experiments were made on male rats, Sprague-Dawley, 150–180 g, and mice, NMRI 20–22 g caged in groups of three. To standardize dietary intake of tryptophan, food was withheld from all animals 12 h before the experiments which were run at an ambient temperature of 22–23°.

Drugs used were: (±)-amphetamine sulphate, (+)-amphetamine sulphate, *p*-chloroamphetamine hydrochloride, *p*-hydroxyamphetamine hydrochloride, nialamide (Niamid, Pfizer) and α-propyldopacetamide (H 22/54, Hässle). Doses in the Tables and Figures refer to the bases of the drugs.

[^{3}H]-l-Tryptophan (7·3Ci/mM, uniformly labelled, New England Nuclear Corp.) was dissolved in 0·9% saline (200 μCi/ml) and injected into a tail vein of the animals in restraining cages. At various time intervals after administration of the drugs the animals were stunned and decapitated. The brain, and a part of the liver were rapidly removed and cooled on ice. Blood was collected from the vena cava of rats and from the carotid artery of mice.

Isolation of tryptophan

Serum (0·2–0·5 ml), brain and liver were homogenized in 10 ml of ice-cold 0·4N perchloric acid (containing ascorbic acid, 0·2 mg/ml) using a glass homogenizer.

Tryptophan was isolated by ion-exchange chromatography. After centrifugation of the homogenate, the pH was adjusted to 6·5 with 5N K_2CO_3. Precipitated potassium perchlorate was removed by centrifugation. Indole amines were removed by passing the extract through a column of Amberlite CG 50 type I (6 × 30 mm, K^+-form). The pH of the effluent was adjusted to 2·2 with HCl and the solution was poured onto a column of Dowex 50W–X4 (6 × 35 mm, K^+-form). After rinsing the column with 5 ml of H_2O and 3 ml of 0·1N phosphate buffer, pH 6·5, tryptophan was eluted with another 15 ml of the buffer (Dowex eluate).

Determination of tryptophan

Two methods were used for estimating the content of tryptophan in the Dowex eluates. Where not otherwise stated tryptophan determinations were made fluorimetrically.

Fluorimetric method. Condensation of tryptamine or tryptophan with formaldehyde and subsequent dehydrogenation results in formation of the highly fluorescent compound norharmane. The fluorescence intensity is proportional to the content of tryptamine and tryptophan in the extract (Hess & Udenfriend, 1959). Since tryptamine is removed by ion-exchange chromatography, norharmane fluorescence in the Dowex eluate should represent tryptophan. The original method (Hess & Udenfriend, 1959) was slightly modified, i.e. internal tryptophan standards were used and tissue blanks were prepared by adding H_2O_2 before formaldehyde and by omitting the incubation step. Fluorescence and activation spectra of extracts of saline- and (±)-amphetamine-treated animals were compared with those from authentic tryptophan and were found to have similar maxima: activation wavelength 365 nm; fluorescence wavelength 440 nm (uncorrected instrumental values, Aminco-Bowman spectrophotofluorometer). The mean recovery of small amounts of labelled and unlabelled tryptophan added to tissue extracts and passed through the whole procedure was 70%. The experimental error of the method (s.d.) for tryptophan determination estimated from ten double samples of serum was 7%.

Microbiological method. The highly specific cup-plate assay of L-tryptophan introduced by Bolinder (1969) was used. Aliquots of Dowex eluates or serum were incubated in a tryptophan-free medium containing lactic acid bacteria. The growth of the bacteria is proportional to the content of L-tryptophan of the extract. The presence of (±)-amphetamine did not interfere with the microbiological assay. These determinations were kindly performed by tekn. lic. Arne E. Bolinder, Tekniska högskolan, Stockholm, Sweden.

Determination of radioactivity

Total radioactivity of tissue extracts or Dowex eluates ([^{3}H]tryptophan, see Results) was determined by dissolving aliquots in Instagel (Packard Instr. Co.) followed by liquid scintillation counting in a Packard Tri-carb spectrometer with absolute activity analyser.

Paper chromatography. A small fraction of the Dowex eluates from the brain extracts of saline-treated animals (EXTR I) and of (±)-amphetamine-treated animals (EXTR II) was applied on Whatman No. 1 filter paper together with reference solutions of anthranilic acid (AA), 5-hydroxyindoleacetic acid (5-HIAA), indoleacetic acid (IA), indoxyl sulphate (IS), kynurenine (KY), kynurenic acid (KA), tryptophan (TP) and xanthurenic acid (XA), 10 μg of each. The chromatograms were run in two solvent systems: potassium chloride (8% w/v, ascending) for 6 h or n-butanol : acetic acid : water (12 : 3 : 5, ascending) for 12 h. After drying the chromatograms the reference spots were visualized in ultraviolet-light or by spraying the chromatograms with Ehrlich reagent (Jepson, 1960). The localization of radioactivity was determined by cutting the chromatograms in 1 cm strips. These were eluated with 3 ml of N HCl, dissolved in Instagel and counted in vials by liquid scintillation spectrometry (Fig. 1). Practically all the radioactivity in the Dowex

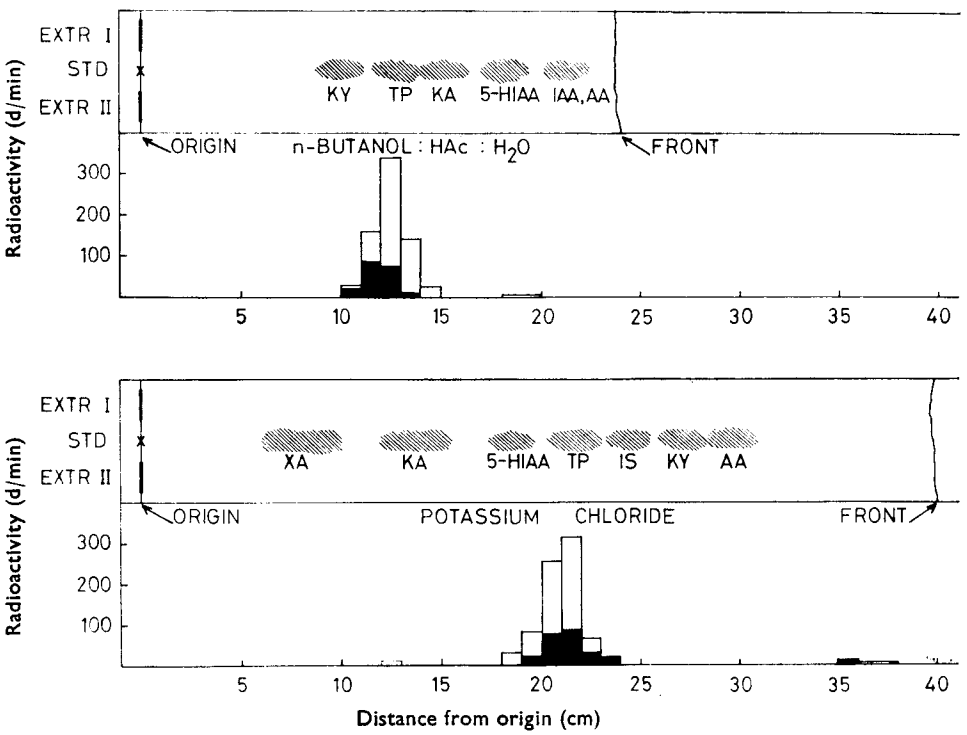

FIG. 1. Paper chromatographic analysis of [^{3}H]tryptophan encountered in Dowex eluates of brain extracts from saline-treated (black columns) and (±)-amphetamine-treated (white columns) mice. [^{3}H]Tryptophan was injected i.v. 20 min after i.p. administration of saline or (±)-amphetamine (10 mg/kg). Animals were killed 80 min after injection of the drugs. The solvents were potassium chloride (8% w/v) and n-butanol–acetic acid–water (12 : 3 : 5). The chromatograms were processed by ascending chromatography for 6 and 12 h respectively. Reference markers were anthranilic acid (AA), 5-hydroxyindoleacetic acid (5-HIAA), indoleacetic acid (IA), indoxyl sulphate (IS), kynurenine (KY), kynurenic acid (KA), tryptophan (TP) and xanthurenic acid (XA). (See Methods.)

eluates from saline- as well as (±)-amphetamine-treated animals migrated as authentic trytophan.

RESULTS

Effect of (±)-amphetamine on the content of tryptophan in brain and serum of mice and rats

Mice were injected intraperitoneally with saline or (±)-amphetamine (10 mg/kg) 120 and 60 min before death. Tryptophan in brain and serum was isolated and measured by the methods described, similar results were obtained with both (Table 1). (±)-Amphetamine treatment resulted in an approximately three-fold increase in the tryptophan content in brain and an almost two-fold increase in serum. The tryptophan content in serum was also measured microbiologically after dilution with distilled water omitting the ion-exchange chromatographic purification. Tryptophan content after (±)-amphetamine treatment was also found to be increased (Table 1, figures in brackets).

Table 1. *Endogenous concentrations of tryptophan in brain and serum of mice after treatment with (±)-amphetamine determined with fluorimetric and microbiological methods.* Saline or amphetamine (10 mg/kg) was administered i.p. 120 and 60 min before death. The content of tryptophan was determined in Dowex eluate of tissue extracts and in non-purified serum (figures in brackets). Figures represent mean value ± s.e. from 5 animals.

	Tryptophan (μg/g or ml)			
	Fluorimetric (Hess and Udenfriend, 1959)		Microbiological (Bolinder, 1969)	
Treatment	Brain	Serum	Brain	Serum
Saline	3·9 ± 0·5	7·4 ± 0·6	4·7 ± 0·2	11·5 ± 0·8 (12·9 ± 0·4)
Amphetamine ..	12·6 ± 1·8*	12·0 ± 1·2*	9·9 ± 0·8*	17·2 ± 1·4* (16·7 ± 0·9*)

* Differs from saline group, $P<0·001$.

Similar experiments in rats showed that (±)-amphetamine (10 mg/kg) significantly ($P<0·001$) elevated the content of tryptophan in brain from 2·5 ± 0·5 to 7·7 ± 1·5 μg/g and in serum from 11·0 ± 1·6 to 18·4 ± 1·9 μg/ml (means ± s.e. of 4–5 rats, fluorimetric assay).

Tryptophan concentrations in brain, serum and liver of mice after treatment with (±)-amphetamine

(±)-Amphetamine (10 mg/kg) or saline was administered intraperitoneally to mice 90 min before death. Serum was collected and the brain and liver were rapidly removed and analysed for tryptophan. (±)-Amphetamine elevated the tryptophan concentration in brain and serum, but not in the liver, when compared to controls. The tryptophan content (μg/g or ml) in brain being 4·5 ± 0·5 (saline), 8·1 ± 0·6 (amphetamine), $P<0·001$; in serum 6·2 ± 0·9 (saline), 9·6 ± 1·1 (amphetamine), $P < 0·05$; in liver 6·1 ± 0·6 (saline), 6·0 ± 0·8 (amphetamine) (means ± s.e. of 6 mice).

[^{3}H] Tryptophan (100 μCi) was injected intravenously 20 min after saline or

(±)-amphetamine (10 mg/kg). The animals were killed 1 h after the labelled tryptophan was given and its content in brain and liver was determined. (±)-Amphetamine significantly elevated the [^{3}H]tryptophan concentration in both brain and liver (Table 2).

Table 2. *Effect of (±)-amphetamine on the content of labelled tryptophan in brain and liver of mice.* [^{3}H]Tryptophan (100 μCi) was injected i.v. 20 min after administration of saline or (±)-amphetamine (10 mg/kg, i.p.). Animals were killed 1 h after administration of [^{3}H]tryptophan. Figures represent mean value ± s.e. from 6–8 animals.

	[^{3}H]Tryptophan (d/min 10^{-3}/g)		Tryptophan (μg/g)	
Treatment	Brain	Liver	Brain	Liver
Saline	23 ± 0·7	58 ± 4·8	3·5 ± 0·2	4·9 ± 0·8
Amphetamine	70 ± 4·7*	140 ± 15·5*	7·3 ± 0·6*	5·2 ± 0·7

* Differs from saline group, $P<0{\cdot}001$.

Effect of the dose of (±)-amphetamine on the elevation of tryptophan concentrations in mouse brain

Groups of mice were injected with (±)-amphetamine in doses from 1 to 30 mg/kg (i.p.). Sixty min later the animals were killed and the tryptophan content in brain was measured (Fig. 2). Even with the smallest dose, a significant ($P<0{\cdot}01$) elevation of the tryptophan content was obtained. The effect increased with increasing dosage.

Time course for the effect of (±)-amphetamine on the contents of labelled and endogenous tryptophan in mouse brain

Mice were treated with saline or (±)-amphetamine (10 mg/kg, i.p.) 20 min before an injection of [^{3}H]tryptophan (100 μCi, i.v.). Groups of animals were killed at different time intervals between 2·5 and 240 min after the labelled tryptophan was

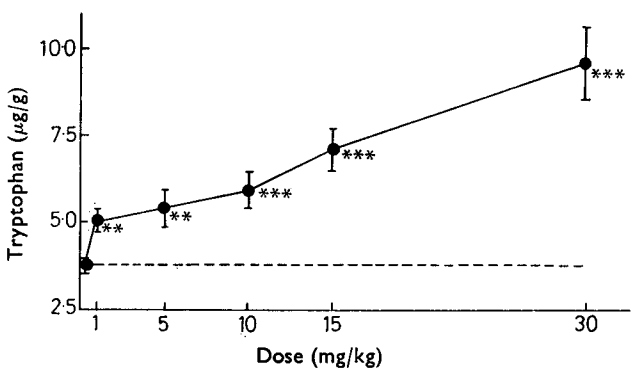

FIG. 2. Effect of dose of (±)-amphetamine on the content of tryptophan in mouse brain. (±)-Amphetamine (———) or saline (– – – –) was administered i.p. 1 h before death. Each point is the mean ± s.e. of 6 animals. *** Differs from saline group, $P<0{\cdot}001$. ** Differs from saline group, $P<0{\cdot}01$.

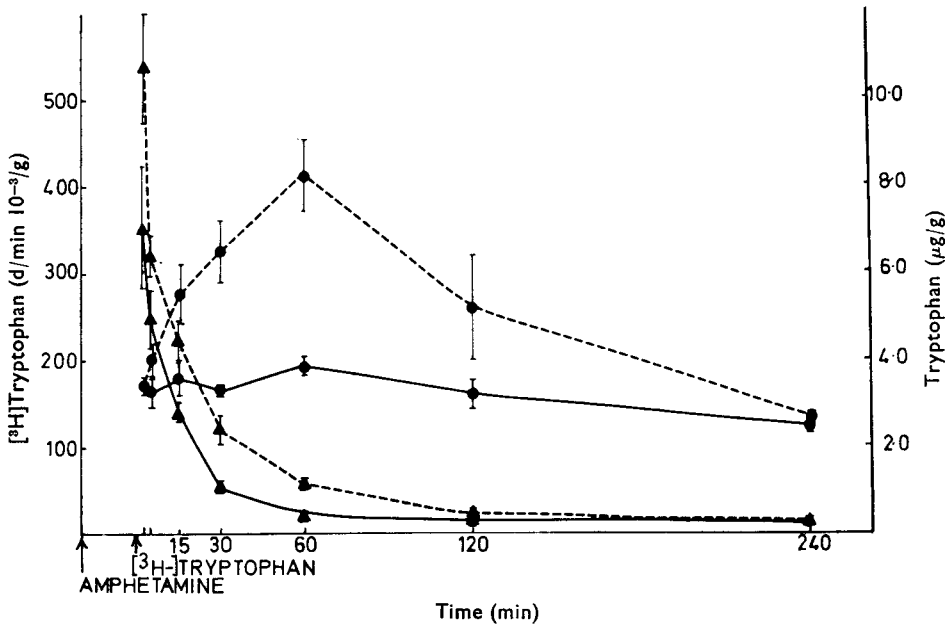

FIG. 3. Effect of (±)-amphetamine (10 mg/kg) on the content of labelled (▲) and endogenous (●) tryptophan in mouse brain at various time intervals after i.v. injection of [³H]tryptophan (100 μCi). Saline (———) or (±)-amphetamine (– – – –) was administered i.p. 20 min before [³H]tryptophan. Each point is the mean ± s.e. of 4 or 5 animals.

given. Brains were analysed for labelled and endogenous tryptophan (Fig. 3). (±)-Amphetamine seemed to enhance the accumulation of [³H]tryptophan in brain and the concentration of [³H]tryptophan remained higher in (±)-amphetamine-treated animals than in controls for at least 60 min after administration of the tryptophan.

The concentration of endogenous tryptophan was not altered significantly in saline-treated animals after injection of [³H]tryptophan (Fig. 3). After (±)-amphetamine treatment, however, there was a slow rise in the content that reached a maximum 80 min after (±)-amphetamine administration. After 260 min the brain content of tryptophan was back at the control level.

Effect of inhibition of monoamine oxidase and tryptophan hydroxylase on the content of tryptophan in mouse brain

Mice were treated with saline, nialamide (100 mg/kg) or (±)-amphetamine (10 mg/kg). Sixty min later animals were killed and the content of tryptophan in brain was determined (Table 3). (±)-Amphetamine but not nialamide increased the content significantly.

In a separate experiment, saline, (±)-amphetamine (10 mg/kg), the tryptophan hydroxylase inhibitor H 22/54 (500 mg/kg) or both the latter drugs were administered to mice 60 min before death. H 22/54 had no significant influence on the tryptophan content in brain and did not affect the increase of the tryptophan concentration produced by (±)-amphetamine (Table 3).

Table 3. *Tryptophan concentration in brains of mice after treatment with (±)-amphetamine, nialamide or H 22/54.* Saline, amphetamine, nialamide, H 22/54 or amphetamine + H 22/54 was administered i.p. 60 min before death. Mean ± s.e. from 4–7 animals.

Treatment	Dose mg/kg	Tryptophan μg/g
Saline	—	2·5 ± 0·1
Amphetamine	10	7·7 ± 1·5*
Nialamide	100	3·0 ± 0·3
Saline	—	4·0 ± 0·2
Amphetamine	10	7·2 ± 0·6**
H 22/54	500	3·3 ± 0·3
Amphetamine + H 22/54	10 + 500	5·8 ± 0·6**

* Differs from saline group, $P<0·001$.
** Differs from saline group, $P<0·01$.

Effect of some amphetamine derivatives on the content of tryptophan in mouse brain

Groups of mice were treated with (+)-amphetamine, (±)-amphetamine, *p*-chloroamphetamine or *p*-hydroxyamphetamine (all at 10 mg/kg, i.p.). The animals were killed 1 h after treatment and their brains analysed for tryptophan. All drugs significantly increased the tryptophan concentration in brain compared to controls. (+)-Amphetamine had an effect significantly greater than that of *p*-chloroamphetamine and *p*-hydroxyamphetamine but not significantly different from (±)-amphetamine (Table 4).

Table 4. *Effect of some amphetamine derivatives on the concentration of tryptophan in mouse brain.* Animals were killed 1 h after i.p. injection of saline or drugs. Figures represent mean value ± s.e. from 6–10 animals.

Treatment	Dose mg/kg	Tryptophan (μg/g)	
Saline	—	3·4 ± 0·2	
(+)-Amphetamine	10	8·5 ± 0·7*	
(±)-Amphetamine	10	6·4 ± 0·7*	
p-Chloroamphetamine	10	5·1 ± 0·2*	**
p-Hydroxyamphetamine	10	4·5 ± 0·2*	**

* Differs from saline group, $P<0·001$.
** Differs from (+)-amphetamine group, $P<0·005$.

DISCUSSION

We found earlier that after treatment with (±)-amphetamine the tryptophan concentration in the mouse brain was increased (Schubert & others, 1970). Three independent methods were used to demonstrate the effect of (±)-amphetamine on the tryptophan concentrations in rats and mice. The fluorescence data and the results obtained with the highly specific microbiological method were consistent and demonstrate that the concentrations of endogenous tryptophan in brain and serum are elevated after (±)-amphetamine treatment (Table 1). Moreover, pretreatment of the animals with (±)-amphetamine seemed to enhance the accumulation and

significantly retarded the disappearance of intravenously administered [^{3}H]tryptophan from brain (Figs 1 and 3).

The amphetamine-induced elevation of the tryptophan concentration in brain is dose-dependent (Fig. 2). The tryptophan content in brain was significantly elevated with a dose of 1 mg/kg of (±)-amphetamine, a dose that causes motor stimulation in rats (Stolk & Rech, 1967).

The endogenous concentration of tryptophan was significantly elevated in brain and serum but not in the liver (Tables 1 and 2). However, since the content of [^{3}H]tryptophan in the liver as well as in brain 1 h after its intravenous administration was higher in amphetamine-treated animals than in controls (Table 2), the drug has an effect in all the three tissues. Hepatic concentrations of endogenous tryptophan are maintained relatively constant when compared with plasma concentrations, presumably owing to induction of regulatory catabolic enzymes in the liver (Francesconi & Mager, 1971).

To ascertain if amphetamine itself or some of its metabolites causes the effect on the tryptophan concentrations, the drug was administered both to rats and mice as these have different metabolic patterns for the degradation of amphetamine. In the rat the major reaction is aromatic hydroxylation whereas in the mouse hydroxylation and oxidative deamination are equally represented (Smith & Dring, 1970). In both species (±)-amphetamine elevated the concentrations of tryptophan in brain and serum to about the same extent, indicating that the metabolic fate of amphetamine is probably of minor importance regarding the above effect.

After injection of (±)-amphetamine the endogenous concentration of tryptophan in brain increased relatively slowly, to reach maximum about 80 min after drug administration (Fig. 3). Since tryptophan is not synthesized in mammalian tissue, the increase could be due to (1) interference with uptake or transport of tryptophan, (2) an impaired binding of tryptophan and/or (3) blockade of metabolic pathways for the amino-acid.

To determine how amphetamine influences the kinetics of intravenously administered tryptophan, the accumulation and disappearance of labelled tryptophan was examined (Fig. 3). The rapid and high accumulation of [^{3}H]tryptophan in brain after pre-treatment of the animals with (±)-amphetamine supports the possibility of either a displacement of bound forms of tryptophan or that amphetamine might affect amino-acid uptake and transport resulting in excess of tryptophan extracellularly and in plasma (Guroff & Udenfriend, 1962; Grahame-Smith & Parfitt, 1970).

The finding that the concentration of [^{3}H]tryptophan in brain of (±)-amphetamine-treated animals was higher than controls during the first hour after [^{3}H]tryptophan injection, could indicate a block of tryptophan degradation by amphetamine. Such an effect could also explain the relatively slow increase of the endogenous tryptophan concentrations. Since amphetamine is a weak inhibitor of monoamine oxidase and since one minor pathway of tryptophan metabolism involves 5-hydroxylation, the effect of nialamide, a potent monoamine oxidase inhibitor and H 22/54, a tryptophan hydroxylase inhibitor (Carlsson, Corrodi & Waldeck, 1963), on the tryptophan concentrations in brain were tested. Since both agents were without effect (Table 3), it is unlikely that amphetamine elevates tryptophan concentrations in the tissues by inhibition of monoamine oxidase or tryptophan hydroxylase.

The major route for tryptophan catabolism is the kynurenine pathway involving

the tryptophan pyrrolase in the liver. Since pyrrolase is the rate-limiting enzyme in the conversion of tryptophan to kynurenine (Yamagushi, Shimoyama & Gholson, 1967; Sourkes, Missala & Madras, 1968) blockade of this metabolic step might also give rise to the increase of labelled and endogenous tryptophan concentrations reported in this paper. However, preliminary results on the effect of (±)-amphetamine on pyrrolase activity in the liver have shown that the drug does not inhibit this enzyme after drug administration (Schubert, in preparation).

It seems unlikely that the observed effects of (±)-amphetamine on tryptophan concentrations are mediated via some general stress mechanism. Stress increases adrenal hormone secretion which in its turn induces tryptophan pyrrolase activity (Civen & Knox, 1960; Lee & Baltz, 1962) and tryptophan hydroxylase activity (Azmitia & McEwen, 1969). However, if amphetamine induces catabolic enzymes which, in fact, it seems to do (Schubert, in preparation), the tryptophan concentration in tissues would be decreased, which is contrary to the present results. This indicated that blockade of metabolic pathways for the amino-acid is not the mechanism for the amphetamine-induced elevation of tryptophan content in tissues. An effect of amphetamine on tryptophan binding and/or transport seems more likely.

Among different amphetamine analogues tested for effect on the tryptophan concentration in brain, (+)-amphetamine and (±)-amphetamine were the most potent (Table 4). *p*-Chloroamphetamine and *p*-hydroxyamphetamine, which have weak or no central stimulant properties compared with (+)-amphetamine (Kaergaard, Nielsen & others, 1967), had significantly less effect on the content of tryptophan in brain than (+)-amphetamine. These findings might indicate that amphetamine-induced changes in tryptophan concentrations in tissues are related to the central stimulant effect of the drug used. This is of interest since tryptophan has been suggested to have antidepressant properties (Coppen, Shaw & others, 1967; Broadhurst, 1970).

Since tryptophan is the precursor of 5-hydroxytryptamine (5-HT) and changes of the amino-acid concentration in tissues has been shown to influence 5-HT concentrations in brain (Fernstrom & Wurtman, 1971; Grahame-Smith, 1971), the effects of amphetamine on tryptophan concentrations reported here presumably influences 5-HT synthesis and turnover in brain and other tissues (Schubert & others, 1970; Tonge & Leonard, 1970; Tagliamonte, Tagliamonte & others, 1971).

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