gave similar results to those obtained with immunoelectrophoresis.  It allows for the rapid processing of a large number of determinations.  It should be useful for the analysis of small samples such as cultured cells, biopsies, etc.  It might also be used for kinetic studies of induction in vivo or with cultured cells.  Such studies are now in progress in our laboratory, in parallel with the development of other monoclonal or polyclonal antibodies for the assay of other cytochrome P-450 isoenzymes.

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AMINO ACID DISTRIBUTION IN BRAIN AFTER USE OF
AMPHETAMINES AND β-PHENYLETHYLAMINE

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SUMMARY:  Amino acids in rat brain were assayed after IP injection d-am-
phetamine or β-phenylethylamine (PEA).  Results revealed elevated values
when one utilized 2.0-12 mg/kg of d-amphetamine.  At 15 mg/kg, however, all
amino acids fell into the control range except tryptophan which was elevated
nearly threefold, and methionine which showed a tenfold decrease.  When
utilizing PEA to induce the behavioral changes only methionine is decreased
at all concentrations of PEA.  Chlorpromazine did not disturb the amino acid
distribution induced by amphetamine or PEA.  When haloperidol was utilized
as the neuroleptic to prevent behavioral change there was a significant
increase above control of all the amino acids including homocysteine.  The
implications of this are discussed in the text.

It has been established that amphetamines or β-phenylethylamine (PEA)

can induce a stereotypic behavior pattern in rats, as well as cause disaggre-

gation of polyribosomes and inhibition of protein synthesis (1-3) in the

acutely treated rat.

Weiss et al (4) have reported an elevation of tryptophan levels in

brain after injection of L-DOPA which has also been shown to disaggregate

polyribosomes.  Aoki and Siegel (5) exploring causes of brain damage in

phenylketonuria found that injection of phenylalanine into young rats induced

polyribosomal disaggregation in brain but not in liver, and resulted in

significant reduction in brain levels of tryptophan.  The polyribosome

disaggregation paralleled the depletion of brain tryptophan levels.

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Because of possibly important effects on behavior and protein synthesis

in rat brain we have investigated the concentrations of amino acids in whole

brain of rats following injection of different doses of d-amphetamine and

PEA.  The level of amino acids was also determined in the presence of the

neuroleptics haloperidol and chlorpromazine which inhibit abnormal behavioral

manifestations caused by injecting amphetamine or PEA.

## MATERIALS AND METHODS

Animals.  CD rats weighing between 300-350 g were divided into 12 groups
for treatment purposes.  Rats were housed individually at room temperature
and maintained on a 12:12 h (7 a.m.-7 p.m.) light/dark schedule with Purina**
Laboratory Chow and water continuously available.  Four groups (total 25
rats) were injected intraperitoneally with d-amphetamine 2, 3.5, 12, 15 mg/kg,
respectively.  Nine rats, three per dosage level, were injected (IP) with
25, 50, and 100 mg/kg of PEA, respectively.  Two groups of rats received the
neuroleptic chlorpromazine 15 mg/kg prior to the injection of either d-amphetamine
15 mg/kg (3 rats) or β-PEA 100 mg/kg (2 rats) while haloperidol 5 mg/kg was
given IP to one group before injecting 15 mg/kg of d-amphetamine (2 rats).
The neuroleptics were given to protect against the abnormal behavioral
manifestations observed with high levels of injected PEA or amphetamine.
Two groups (5 rats) were used as controls.
      Behavior of the animals was observed for up to 1 h after the injection.
Exhibition of stereotypic effects generally occurred within 20 min with
amphetamine and 5-10 min with PEA.  The animals given the neuroleptics
before amphetamine or PEA were observed for 1/2 h.  Following this period of
observation the animals were decapitated in a cold room (4°C) and the brain
rapidly removed and washed with physiological saline until free of blood.
The brains were than prepared for amino acid analysis.

      Amino acid analysis.  The flow chart of the procedure for extraction
and concentration of amino acids is shown (Fig. 1).  Brains of treated and
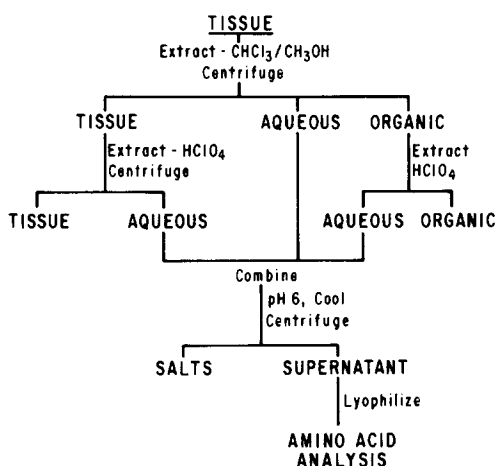control animals were pooled for amino acid analysis.



Fig. 1 - Flow Chart of Procedure for Extraction of Amino Acids.

Brains were macerated and extracted in a blender (Waring, Model 91-262) containing 20 ml of Folch's reagent (chloroform/methanol 2:1) at high speed for 1 min.  The slurry was centrifuged and the three layers (tissue, chloroform (organic) layer, and upper aqueous phases) were separated.  The tissue residue was re-extracted 2 X with 20 ml Folch's reagent and the supernatants combined.  The combined chloroform layers were extracted 3 X with 100 ml of 0.6 M perchloric acid and centrifuged.  The layers were separated and the tissue re-extracted twice more.  Perchloric acid extracts and the initial aqueous phases combined and adjusted to pH 6 with KOH and cooled.  The precipitate was washed with ice cold deionized water and the washings were added to the supernatant.  The supernatant was concentrated by freeze drying and redissolved in 10 ml of sodium citrate buffer, pH 2.2.  Amino acids were separated by using a Beckman Model 119-B and 119-CL A. A. Analyzer pack with resins AA-20 and W-3, respectively.  The brains were dissolved in sodium citrate buffer pH 2.2 and separated with the following buffers of pH 3.25, 3.95, and 6.4.

## RESULTS

Amphetamines.  Nineteen free amino acids were determined in whole rat brain following the injection of varying concentrations of amphetamine.  All the amino acids gave evidence of a quadradic ($p < 0.05$) relationship with increased levels of amphetamine except lysine, valine, and proline (Table 1).

Table 1

Effect of IP Administration of d-Amphetamine on Free Amino Acids in Rat Brain

Concentration of Free Amino Acids (nM/g Tissue)

| Amino Acids | Control | | 2 mg | | 3.5 mg | | 12 mg | | 15 mg | |
|---|---|---|---|---|---|---|---|---|---|---|
| Aspartic acid | 1062 | 927 | 8671 | 8489 | 10,414 | 10,092 | 10,398 | 9375 | 1976 | 2004 |
| Tryptophan | 9 | 8 | 97 | 67 | 128 | 117 | 108 | 51 | 26 | -- |
| Threonine | 374 | 349 | 2329 | 2204 | 5830 | 2754 | 2629 | 2386 | 475 | 475 |
| Serine | 1023 | 900 | 7494 | 7304 | 7718 | 7737 | 8487 | 7527 | 1612 | 1610 |
| Glutamic acid | 425 | 900 | 4179 | 4092 | 4101 | 4242 | 5162 | 4255 | 1010 | 908 |
| Proline | 765 | 286 | 1575 | 1489 | 528 | 1707 | 1940 | -- | -- | -- |
| Glycine | 1009 | 680 | 4877 | 4600 | 5287 | 5287 | 5028 | 4641 | 1148 | 1169 |
| Alanine | 1788 | -- | 3468 | 3413 | 4503 | 4235 | 3439 | 3572 | 923 | -- |
| Valine | 550 | 950 | 1874 | 1650 | 1902 | 1879 | 1549 | 2066 | -- | -- |
| Methionine | 466 | 311 | 396 | 389 | 400 | 938 | 536 | 637 | 45 | -- |
| Isoleucine | 177 | 390 | 808 | 901 | 1057 | 1344 | 1041 | 957 | 196 | 200 |
| Leucine | 426 | 889 | 1754 | 2168 | 2466 | 2555 | 2540 | 2325 | 480 | 487 |
| Tyrosine | 1502 | 4396 | 6673 | 6862 | 7493 | 6804 | 9578 | 9037 | 3034 | 3108 |
| Phenylalanine | 430 | 467 | 1144 | 1319 | 1901 | 1501 | 1661 | 1936 | 500 | 454 |
| Hydroxylysine | 685 | 855 | 2204 | 2142 | 2413 | 2024 | 1072 | 1097 | 399 | 434 |
| Lysine | 751 | 1077 | 2349 | 5361 | 2936 | 2687 | 2736 | 2588 | 742 | 781 |
| Histidine | 279 | 401 | 1057 | 1074 | 1257 | 1128 | 1188 | 1079 | 296 | 351 |
| Arginine | 350 | 610 | 1655 | 1643 | 1818 | 1712 | 1973 | 1786 | 300 | 384 |
| Cysteine-Cystine | 955 | 1258 | 1004 | 751 | 1693 | 1734 | 1708 | 1940 | 684 | 680 |

The administration of 15 mg/kg of amphetamine reversed this trend and depressed the concentration of free amino acids close to or below the level measured in the controls.

At 2 mg/kg all the amino acids were elevated 200%-800% above the control with the exception of methionine where the value remained in the control range as was the case for cysteine-cystine. At 3.5 mg/kg all the amino acids levels increased 150 to 1000% over control except methionine, which was equal to or underwent an increase in one experiment of 100%. The data at the 12 mg/kg level is similar as all amino acids increase 150%-1000% while methionine only increased 50%-100%.

Administration of amphetamine at the 15 mg/kg level caused marked reduction in free amino acids for the most part when compared to administration with lower doses (2-12 mg/kg amphetamine). Aspartic acid, tryptophan, threonine, serine, and glutamic acid are increased only twofold while iso-leucine, leucine, tyrosine, alanine, lysine, histamine, and arginine remained at the same level as the control. Three amino acids including alanine, hydroxylysine, and methionine decreased with methionine undergoing a tenfold reduction. At 2-12 mg/kg methionine remains unchanged or undergoes only a modest increase. This has been calculated as 1/4 to 1/20 the increase found in the other amino acids. After injection of 15 mg/kg of amphetamine, however, there is a marked reduction in methionine as seen in Table 1. Whether the amino acids were acid, basic, or neutral did not appear to play a role in the eventual concentration of the amino acids in brain tissue.

PEA. Administration of β-PEA (25, 50, 100 mg/kg) does not significantly effect the free amino acids (Table 2), as did the administration of amphetamine. Only methionine shows evidence of a significant ($p < 0.05$) linear effect due to increased levels of PEA. Methionine is consistently depressed to 40% of the control. Aspartic acid exhibits evidence of similar behavior, but only at a lower significance (<0.10) level. An increase in cysteine-cystine[††] occurred after administration of 100 mg/kg of PEA in one experiment.

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[††]With the procedure utilized, one is also measuring homocysteine.

Table 2

Effect of Administration of $\beta$-PEA on Free Amino Acids in Rat Brains

Concentration of Free Amino Acids (nM/g Tissue)

| Amino Acids | I | | | | II | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 25 mg/kg | 50 mg/kg | 100 mg/kg | Control | 25 mg/kg | 50 mg/kg | 100 mg/kg |
| Lysine | 444 | 197 | 98 | 247 | 186 | 317 | 191 | 482 |
| Histidine | 100 | 53 | 69 | -- | -- | 81 | 56 | -- |
| Arginine | 338 | 249 | 291 | 117 | 441 | 398 | 496 | 452 |
| Aspartic acid | 1994 | 1550 | 2151 | 1599 | 2404 | 2269 | 2429 | 1846 |
| Threonine | 3758 | 2669 | 2801 | 3236 | 4971 | 5318 | 5485 | 4615 |
| Glutamic acid | 2876 | 2330 | 3069 | 1895 | 3934 | 4907 | 4347 | 3761 |
| Glycine | 1250 | 944 | 1420 | 1098 | 1569 | 1674 | 1799 | 1475 |
| Alanine | 394 | 401 | 779 | 644 | 569 | 757 | 816 | 571 |
| Cysteine-cystine | 387 | 124 | 257 | 464 | 399 | 443 | 448 | 1329 |
| Valine | 182 | 155 | 238 | 179 | 218 | 197 | 206 | 169 |
| Methionine | 106 | 60 | 60 | 55 | 117 | 62 | 101 | 71 |
| Isoleucine | 115 | 82 | 131 | 108 | 89 | 113 | 139 | 92 |
| Tyrosine | 131 | 92 | 130 | 106 | 132 | 120 | 144 | 125 |
| Phenylalanine | 132 | 229 | 132 | 123 | 152 | 678 | 177 | 122 |
| Leucine | 323 | 211 | 328 | 272 | 277 | 278 | 343 | 245 |

Neuroleptics.  Injection of neuroleptics to protect the animals against

behavioral aberrations usually induced by amphetamine or PEA gave interesting

results (Table 3).  When chlorpromazine 15 mg/kg was injected prior to

amphetamine (15 mg/kg), abnormal behavioral changes were prevented in all

cases and the amino acids measured in nm/gm brain tisssue generally were in

the range of the controls with the exception of lysine which showed a threefold

elevation.

These values were very similar to those noted when chlorpromazine

(15 mg/kg) was utilized as the protectant against the manifestations with

PEA (100 mg/kg), again with some notable exceptions as shown in Table 3.

Conversely when animals were injected with haloperidol (5 mg/kg) doubling of

the individual amino acid concentration in nm/gm brain tissue over the

controls was noted when compared with the concentration of the amino acids

while protecting the animals with chlorpromazine after the injection of

either amphetamine or PEA (see Table 3).

Table 3

Amino Acid Distribution After the Use of Neuroleptics to Protect Against Stereotypies

Concentration of Free Amino Acids (nM/g Tissue)

| Amino Acid | Amphetamine 15 mg/kg + Control | Amphetamine 15 mg/kg + Chlorpromazine 15 mg/kg | Amphetamine 15 mg/kg + Haloperidol 5 mg/kg | $\beta$ - Phenylethylamine 100 mg/kg + Chlorpromazine 15 mg/kg |
|---|---|---|---|---|
| Lysine | 186 | 548  (2.94) | 411  (2.21) | 181  (0.97) |
| Histidine | -- | -- | -- | -- |
| Arginine | 441 | 391  (0.89) | 873  (1.98) | 336  (0.76) |
| Aspartic acid | 2404 | 2638  (1.10) | 4879  (2.03) | 2596  (1.08) |
| Threonine | 4971 | 5496  (1.10) | 11,044  (2.22) | 5970  (1.20) |
| Glutamic acid | 3934 | 4724  (1.20) | 9347  (2.38) | 5104  (1.30) |
| Glycine | 1569 | 1936  (1.23) | 3281  (2.09) | 2106  (1.34) |
| Alanine | 569 | 920  (1.62) | 1278  (2.25) | 982  (1.73) |
| Cysteine-cystine | 399 | 482  (1.21) | 940  (2.36) | 345  (0.86) |
| Valine | 218 | 178  (0.82) | 398  (1.83) | 178  (0.82) |
| Methionine | 117 | 112  (0.96 | 178  (1.52) | 103  (0.88) |
| Isoleucine | 89 | 119  (1.34) | 241  (2.71) | 80  (0.90) |
| Tyrosine | 132 | 138  (1.05) | 268  (2.03) | 127  (0.96) |
| Phenylalanine | 152 | 155  (1.02) | 291  (1.91) | 105  (0.69) |
| Leucine | 277 | 309  (1.12) | 591  (2.13) | 570  (2.05) |

The number in parenthesis denotes the ratio of the result to controls.

## DISCUSSION

We utilized different amounts of amphetamine and PEA as well as protection against the behavioral manifestation of these amines with the neuroleptics chlorpromazine and haloperidol to determine qualitative and quantitative changes of amino acid distribution in whole brain.

In our work we see a threefold increase in tryptophan and a tenfold decrease in methionine when 15 mg/kg of amphetamine is injected IP in a single dose.  A significant decrease in methionine and an increase in tryptophan in rat brain following a single IP injection of L-DOPA has also been described by Weiss (4).  He suggests that L-DOPA acts on polysomes by limiting the availability of other amino acids such as methionine.  This finding is interesting as methionine is essential in the process of protein initiation.  The methionine as met-tRNA along with GTP and initiation factor EIf-2 form a ternary complex and promotes the formation of a 40 S complex necessary in the eventual initiation and elongation of the polypeptide chain.  It is possible that the

decrease in methionine that we see after the use of 15 mg/kg of amphetamine
IP is the single most important factor in inhibiting protein synthesis.
Baliga and Munro (6) have described a direct inhibitory effect of amphetamine
on peptide chain initiation utilizing natural messenger in vitro through a
step related to formation of the RNA-ribosome complex. They do not elucidate,
however, the specific component affected. Nowak and Munro (7) were more
specific and thought that amphetamine acts on tRNA acylation in inhibiting
protein synthesis. In some of our earlier work utilizing synthetic messenger
in the form of Poly U directed incorporation of tritiated phenylalanine, we
appeared to be affecting the elongation of polypeptides.

In the present investigation the severe depression of methionine concen-
tration in brain after the use of amphetamine and PEA IP suggests that it is
initiation that is affected and may be the major component in the inhibition
of protein synthesis when this drug is employed. When utilizing PEA the
behavioral manifestations are different than with the use of amphetamine.
With PEA we consistently see a Straub tail (the tail is perpendicular to the
longitudinal axis of the animal) and abduction of the hind quarters of the
animal which is very rarely seen with amphetamine. In previous work we have
implicated serotonin as the transmitter probably responsible for these
actions (8) while dopamine appears to incite the behavioral event after the
use of amphetamines. Another interesting finding was at 100 mg/kg PEA we
see nearly a 300% increase in cysteine-cystine (including homocysteine) in
one experiment with only a 25% increase in the second experiment. This
group of amino acids was not affected by injection of amphetamine.

Sprince in 1969 (9) directed attention to the role of homocysteine, a
naturally occurring intermediary metabolite of methionine, as inducing
convulsions. While Sprince used methionine to exacerbate schizophreniform
behavior in animals, we saw a significant decrease in methionine in animals
manifesting stereotypic behavior. One possibility explaining this difference
is a rapid change of gradient across the blood brain barrier and cell membrane
after a large methionine test load was given, while after the induction of

abnormal behavior with amphetamine these changes would be much less rapid allowing more time for adjustment of homostatic mechanisms.

Presently we have no explanation for the very significant elevation of all the amino acids tested when utilizing haloperidol as the neuroleptic protecting against the abnormal behavior after the use of amphetamine. This was not the case when utilizing chlorpromazine as the antipsychotic agent preceding the IP injection of amphetamine. In this case, as shown in the table, all the amino acids tested were comparable to controls except lysine, where we do see a very significant rise of 300%. Utilizing the combination of chlorpromazine and PEA we again see all the amino acids are in the control range. Work is now in progress to elucidate the findings reported in this paper.

## ACKNOWLEDGMENTS

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