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## Effect of Drugs on Amino Acid Levels in Brain: Excitants and Depressants. (26446)

R. S. DE ROPP AND E. H. SNEDEKER (Introduced by B. L. Hutchings)

*Biochemistry Research Dept., Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.*

Previous studies on the action of drugs on amino acid levels in the brain have brought to light a variety of effects. Okumura, *et al.* (1), using ion exchange chromatography to analyze brain amino acids, found that repeated injections of chlorpromazine produced a rise in glutamine, aspartic acid and γ-aminobutyric acid (GABA). Repeated electroshock treatment and repeated injections of β-phenylisopropylmethylamine produced no marked changes in free amino acid levels, although the latter produced an increase in N-acetylaspartic acid. That a reduction occurs in the level of GABA in brains of rats treated with semicarbazide was demonstrated by Killam and Bain(2), but this effect was not observed in animals with seizures induced by pentylenetetrazole. Baxter and Roberts (3) found significant increases in GABA levels in brains of rats treated with hydroxylamine.

The effect of 4 hypoglycemic agents on free amino acid levels in the rat brain has been described(4). The present paper describes results obtained by applying the same analytical procedures(5) to a study of a larger group of agents affecting the central nervous system. These were divided into compounds exerting a predominantly stimulating effect (including the so-called hallucinogens, mescaline and LSD) and compounds exerting a predominantly depressing effect (hypnotics, analgesics and "tranquilizing" agents).

*Experimental.* A. *Design of experiments.* Wistar rats (250 g males) were fasted 24 hours, then injected with the material to be tested dissolved in normal saline. Amounts injected varied and have been recorded under Results. The amount of time allowed for the drug to act also varied. In every experiment 6 groups of 5 rats were used, the experiment being so designed that 2 groups functioned as controls (injected with normal saline), 2 groups received one compound to be tested, and the remaining 2 groups received the second compound to be tested. Thus each experiment consisted of 30 rats distributed as shown below:

| Group | Control | Compound A | Compound B | Total |
|---|---|---|---|---|
| I | 5 | 5 | 5 | 15 |
| II | 5 | 5 | 5 | 15 |
| | 10 | 10 | 10 | 30 |

Each of these unit experiments was repeated 2 or more times, the brains being extracted in one experiment with picric acid and in the other with alcohol. Both extraction procedures and chromatographic methods of determining amino acid levels have been described(5).

Estimations of the significance of treatment effects were made by the method of paired replicates as described by Snedecor (6). This involved obtaining the difference in density reading between each of the treated groups and its own control in each experiment. From the sum of such differences derived from all the experiments carried out, a mean difference was obtained, the

TABLE I. Free Amino Acid Levels (As % of Controls) in Brains of Rats Treated with Agents Having Predominantly Stimulating Effects. (No. of estimates in parentheses.)

| Treatment | Dose, mg/kg | Amino acid | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GSH | Asp. | Glu. | Glu. $NH_2$ | Gly. Ser. | GABA | Ala. | Tau. | Etoh. amine | P. eth. amine |
| Pentylenetetrazole | 100 | 102 ( 6) | 93 ( 9) | 87 (10) | 113 (6) | 109 (2) | 107 (14) | 184 (10) | 102 (5) | 145 (4) | 119 (2) |
| Semicarbazide | 500 | 103 (11) | 81 ( 4) | 97 ( 9) | — | — | 62 (13) | 119 (13) | — | — | 92 (6) |
| Strychnine | 4 | 130 ( 4) | — | 110 ( 4) | — | — | 115 ( 4) | 166 ( 4) | — | — | — |
| Picrotoxin | 12 | 102 ( 8) | 98 (10) | 106 (12) | — | — | 105 ( 9) | 151 ( 9) | 112 (1) | — | 141 (6) |
| Electroshock (convulsion) | — | 104 ( 8) | 95 ( 6) | 109 (10) | 105 (2) | 103 (2) | 109 (10) | 128 (10) | 109 (2) | — | 119 (2) |
| " (recovery) | — | 120 ( 8) | 104 ( 6) | 110 (10) | 115 (2) | 168 (2) | 106 (10) | 131 (10) | 106 (2) | — | 128 (1) |
| Caffeine | 240 | 91 ( 4) | 94 ( 6) | 102 ( 8) | 105 (2) | — | 104 ( 4) | 115 ( 4) | 100 (2) | — | — |
| Pheniprazine | 80 | 88 ( 8) | 103 ( 4) | 103 (10) | 106 (3) | — | 106 ( 8) | 130 ( 8) | 117 (8) | 117 (8) | — |
| Amphetamine | 100 | 110 (20) | 96 (12) | 107 (22) | 108 (5) | — | 83 (18) | 148 (16) | 100 (6) | — | — |
| Mescaline | 240 | 86 (12) | 97 ( 4) | 106 (12) | 108 (2) | — | 108 ( 8) | 130 ( 8) | 90 (2) | — | — |
| LSD | 200 μg | 102 ( 8) | 110 (10) | 101 (10) | 105 (2) | — | 107 (10) | 93 (10) | 100 (6) | — | — |

standard error of which was used to obtain a value for t and a corresponding value for P. Increases or decreases in amino acid levels have been expressed as percentages of the relevant controls and the significance of the changes, calculated as described above, has been recorded. Following accepted usage values of P of .001 or less have been regarded as highly significant, between .01 and .001 as significant and between .05 and .01 as possibly significant.

*Results.* A. *Stimulants.* The effects on brain amino acid levels of 10 agents exerting a predominantly stimulating effect on the central nervous system are shown in Table I. The following clinical effects and significant changes in amino acid levels were observed.

Pentylenetetrazole (100 mg/kg) produced severe convulsions. The rats were killed 30 minutes after injection when these convulsions were at their height. Significant changes in amino acid levels (expressed as percentages of controls) were glutamic, 87% ($P<.01$); glutamine, 113% ($P<.02$); alanine, 184% ($P<.001$). A rise in ethanolamine (145%), though large, was of questionable significance.

Semicarbazide (500 mg/kg) produced convulsions 45 to 60 minutes after treatment. Rats were killed during these convulsions. Significant changes in amino acid levels were GABA, 62% ($P<.001$) and alanine, 119% ($P<.01$).

Strychnine (4 mg/kg) produced severe convulsions 10 minutes after injection. The rats were killed during these convulsions. A significant rise took place in the level of alanine (166%, $P<.01$). A rise in GABA (115%) was of questionable significance.

Picrotoxin (12 mg/kg) produced convulsions during which the animals were killed. The rise in level of alanine was highly significant (151%, $P<.001$). The rise in phosphoethanolamine (141%) was not consistent enough to be significant.

Electroshock (150 mA) was applied directly to the cornea for 0.3 seconds. Rats went into convulsions followed by coma. Half of the rats were killed during the convulsions (about 30 seconds after shock), the other half as they were recovering from the

TABLE II. Free Amino Acids (As % of Controls) in Brains of Rats Treated with Agents Having Predominantly Depressant Effects. (No. of estimates in parentheses.)

| Treatment | Dose, mg/kg | Amino acid | | | | | | | | | Etoh. amine |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GSH | Asp. | Glu. | Glu. $NH_2$ | Gly. Ser. | GABA | Ala. | Tau. | | |
| Meprobamate | 260 | 103 (12) | 109 (10) | 108 (10) | 93 (2) | — | 108 ( 8) | 92 ( 8) | 75 (2) | | 104 ( 6) |
| Chlorpromazine | 100 | 98 (13) | 104 ( 6) | 94 (13) | 141 (8) | — | 105 (13) | 118 (13) | 99 (4) | | 98 (13) |
| Methoxypromazine | 200 | 104 ( 4) | 96 ( 2) | 97 ( 6) | 120 (2) | — | 111 ( 4) | 130 ( 4) | 105 (2) | | — |
| Reserpine | 50 | 91 (13) | 94 (10) | 101 (15) | 103 (2) | — | 108 (15) | 100 (15) | 88 (6) | | — |
| Phenobarbital | 120 | 101 (12) | 93 ( 5) | 86 (15) | 100 (4) | 93 (4) | 88 (13) | 97 (10) | 85 (3) | | 100 ( 5) |
| Chloral hydrate | 240 | 104 ( 4) | 103 ( 2) | 98 ( 4) | 87 (2) | — | 95 ( 4) | 91 ( 4) | 92 (2) | | 85 ( 2) |
| Diphenylhydantoin | 400 | 98 ( 7) | 104 ( 9) | 101 (14) | 102 (8) | 105 (6) | 101 (13) | 113 ( 9) | 97 (8) | | — |
| Acetazolamide | 500 | 92 ( 4) | 99 ( 6) | 84 ( 8) | 103 (2) | — | 82 ( 8) | 97 ( 6) | 98 (2) | | — |
| Morphine | 240 | 87 ( 4) | 76 ( 4) | 71 ( 4) | — | — | 94 ( 4) | 114 ( 4) | — | | — |
| | 10 | — | 108 ( 8) | 108 (16) | 102 (8) | 91 (8) | 110 (16) | 105 (16) | 105 (8) | | — |

coma (about 15 minutes after shock). In the brains of rats killed during convulsions there was a rise in alanine (128%, $P<.02$). In the brains of rats killed during the recovery stage this rise was still present (alanine, 131%). The rises in phosphoethanolamine observed were of doubtful significance.

Caffeine (240 mg/kg) did not produce any obvious excitation in the rats which were killed 1½ hours after injection. A rise in alanine (115%) was observed but its significance was doubtful. Pheniprazine (80 mg/kg) produced excitement in the rats which became extremely vicious. They were killed 2½ hours after injection. A rise in level of alanine to 130% of controls was highly significant (P .001). A rise in level of ethanolamine (117%) was of questionable significance. Amphetamine (100 mg/kg) produced extreme excitement in the rats which were killed while in the excited state. A rise in alanine to 148% of control value was highly significant ($P<.001$). A fall in GABA to 83% was apparently significant (P .01) but considerable variation occurred in the magnitude of this effect in different experiments.

The effects of 2 hallucinogenic drugs on brain amino acid levels were compared. Mescaline (240 mg/kg) produced extreme excitement in the rats which fought so viciously that they had to be separated. They were killed while in the excited state. A rise in alanine (130%) was significant ($P<.01$). A fall in glutathione (86%) was of doubtful significance. Lysergic acid diethylamide (200 μg/kg) did not have an outwardly observable effect on the rats. They were killed 2½ hours after injection. No significant changes in level of their amino acids could be detected.

B. *Depressants.* The effects of 2 hypnotic agents on brain amino acid levels were compared. Phenobarbital (120 mg/kg) reduced the rats to a state of somnolence in which condition they were killed 3½ to 4 hours after injection. A fall in glutamic acid to 86% of controls was significant ($P<.01$) and a fall in level of GABA to 88% was also significant ($P<.01$). Chloral hydrate (240 mg/kg) did not produce observable effects on the rats which were killed 3½ to 4 hours

after injection. No significant changes in brain amino acid levels could be detected in animals treated with this drug.

Acetazolamide (500 mg/kg) did not produce observable physiological effects but lowered the level of brain glutamic acid to 84% of control value ($P<.01$) and lowered GABA level to 82% (P nearly .02). Diphenylhydantoin (400 mg/kg) also produced no observable effect and did not significantly alter levels of brain amino acids.

The effects of 4 tranquilizing agents were compared. Meprobamate (260 mg/kg) produced symptoms of ataxia in the rats which were sacrificed 3½ hours after injection of the drug. No significant changes in levels of amino acids could be detected in the brains of these animals. Chlorpromazine (100 mg/kg) induced a semi-comatose condition in the rats which were sacrificed 4 hours after injection. A rise in glutamine to 141% of control level was highly significant ($P<.001$). A rise in alanine to 118% was also significant (P nearly .001). Methoxypromazine (200 mg/kg) produced drowsiness in the animals which were sacrificed 4 hours after injection. Like chlorpromazine this drug produced a rise in level of brain glutamine (120%) which was significant. It also produced a significant rise in alanine (130%, $P<.01$). Reserpine was administered at the rate of 50 mg/kg per day for 3 days. The animals remained asleep for the last 24 hours and were sacrificed while in that state. No significant changes in brain amino acid levels were observed.

One analgesic, morphine, was tested at 2 dose levels, 240 and 10 mg/kg. At the very high level this drug produced a fall in aspartic acid to 76% of the controls and of glutamic acid to 71%. At the lower dose it did not produce significant changes in brain amino acid levels.

*Discussion.* This study was designed to determine whether agents having in common a certain type of physiological action would also have certain common effects on the metabolism of the brain as reflected in the level of free amino acids in this organ. The 4 convulsant agents tested had one property in common with electroshock. All of them brought about a rise in level of brain alanine ranging from 19% increase produced by semicarbazide to 84% increase produced by pentylenetetrazole. Semicarbazide has already been shown by Killam and Bain(2) to produce a fall in level of GABA in the brain, a finding which is confirmed by the present work. Such a fall in GABA was not produced by the other convulsants tested. It is impossible, for this reason, to attribute the convulsant action of all these agents to a lowering of the level of GABA. Several of these drugs produced rises in level of ethanolamine but because of the low level of this compound in the brain the significance of the rise was questionable.

The excitant drugs, pheniprazine and amphetamine, shared with the convulsant agents the property of increasing brain alanine levels. Caffeine, a milder excitant, did not have this capacity. The hallucinogen, mescaline, produced excitation in the rats and also produced the rise in brain alanine characteristic of excitants. Lysergic acid diethylamide did not produce excitation and had no effect on alanine levels.

The hypnotic agent, phenobarbital, produced a fall in level of both glutamic acid and GABA in the brain, but the chemically unrelated hypnotic, chloral hydrate, did not have this effect. The anti-convulsant, acetazolamide, resembled phenobarbital in producing a fall in level of glutamic acid and GABA, but a second anti-convulsant, diphenylhydantoin, did not have this effect. According to Woodbury and Vernadkis(7) injections of diphenylhydantoin will raise the level of GABA in the rat brain provided the animals have been adrenalectomized. Baxter and Roberts(3) found that "only slight and inconsistent elevations in the GABA levels" were produced in the brains of rats injected with acetazolamide. It does not appear that these anti-convulsants can exert their action by raising GABA levels.

The 2 phenothiazine tranquilizers, chlorpromazine and methoxypromazine both brought about a rise in level of brain glutamine. Okumura *et al.*(1) using chlorpromazine also detected such a rise. A rise in brain alanine was also produced by these 2 sub-

stances. Neither reserpine nor meprobamate produced significant change in level of brain amino acids. The analgesic, morphine, though it produced a lowering in aspartic and glutamic acid levels when an excessively high dose was used, did not exert any effect at a dose level which was still quite adequate to produce analgesia.

Thus, the only correlation between pharmacological activity and biochemical action which emerges from this work is that between the rise in brain alanine and convulsant or excitant activity. Even this is not clear cut because the two phenothiazine tranquilizers tested both produced a rise in alanine levels in the brain although they are not excitants. The rise in glutamine produced by chlorpromazine and methoxypromazine may be characteristic of phenothiazines in general but can hardly be correlated with tranquilizing activity as neither meprobamate nor reserpine exerted this effect.

*Summary.* 1. Effect of electroshock and 18 chemical agents (pentylenetetrazole, semicarbazide, strychnine, picrotoxin, caffeine, pheniprazine, amphetamine, mescaline, LSD, phenobarbital, chloral hydrate, acetazolamide, diphenylhydantoin, meprobamate, chlorpromazine, methoxypromazine, reserpine and morphine) was determined on the level of 10 ninhydrin-reacting compounds in the rat brain (glutathione, phosphoethanolamine, aspartic acid, glutamic acid, GABA, glutamine, glycine +- serine, alanine, taurine and ethanolamine). 2. The convulsant agents, pentylenetetrazole, semicarbazide, strychnine, picrotoxin and electroshock as well as the excitants, pheniprazine, amphetamine and mescaline, brought about significant rises in free alanine in the brain. Lowering of the GABA levels to 60% of that of the controls was brought about by semicarbazide but not by any of the other convulsant agents. 3. Two phenothiazine tranquilizers chlorpromazine and methoxypromazine, brought about a significant rise in level of brain glutamine.

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## Effects of Quinidine on Triacetin Utilizing Activity of Pancreas.* (26447)

GERALD LITWACK AND PEARLEE SHRAGER

*Biochemical Laboratory, Division of Cardiology, Philadelphia General Hospital and Graduate School of Medicine, University of Pennsylvania, Philadelphia*

Rona and Reinicke(1) demonstrated that serum lipase, using tributyrin as substrate, was inhibited by quinine such that the logarithm of the inhibitor concentration plotted against the velocity of the reaction produced a straight line. A protective effect of tributyrin was not observed. Later, Hellerman *et al.*(2) mentioned that both quinine and atabrine inhibited pancreatic lipase reversibly, although no data were presented.

No reports have been seen in the literature which describe the action of quinidine upon triacetin utilizing activity (TUA). Quinidine, a stereosomer of quinine, appears to differ from quinine only in the spatial arrangement of the asymmetric carbon 3, the arrangements of the asymmetric centers, carbons 1, 2 and 4 apparently being identical (3),

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