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Influence of Alkylamides of Glutamic Acid and Related Compounds on the Central Nervous System. II.[1] Syntheses of Amides of Glutamic Acid and Related Compounds, and Their Effects on the Central Nervous System

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Eleven kinds of 5-alkylamides of L- and DL-glutamic acid were synthesized and assessed for anticonvulsant effects. Against lethal convulsion induced by caffeine, maximum efficacy was observed with alkylamides possessing C_7 to C_8 alkyl radicals in the position 5 of glutamic acid, and the compounds having C_5 to C_7 alkyl groups were shown to have the highest toxity. Differences in the response between L-compound and DL-compound are not significant. Synthesized ω-alkylamides of L-glutamic acid, L-aspartic acid and DL-2-aminoadipic acid did neither increase nor decrease the oxygen consumption by the rat brain cortex slices.

In the preceding study[1] we examined the effects of theanine (L-N-ethylglutamine, L-GluNHEt), a constituent of japanese green tea, and L-glutamine (L-GluNH$_2$) on a series of central stimulant actions, the uptakes by brain tissues and influences upon the cerebral free amino acid pattern. The results of the study showed that L-GluNHEt was capable of inhibiting the central stimulant effect of caffeine and the action was stronger than that of L-GluNH$_2$.

Thus the syntheses of several kinds of ω-alkylamides of glutamic acid, aspartic acid and 2-aminoadipic acid were attempted and the compounds synthesized were assessed for anticonvulsant effects and influences on the cerebrocortical respiration in an attempt to clarify interrelations between the chemical structures and the central pharmacological actions of those compounds.

L-N-Alkylglutamines were synthesized from 5-methyl-L-glutamate (I) according to the method of Furuyama, *et al.*[3] (Chart 1).

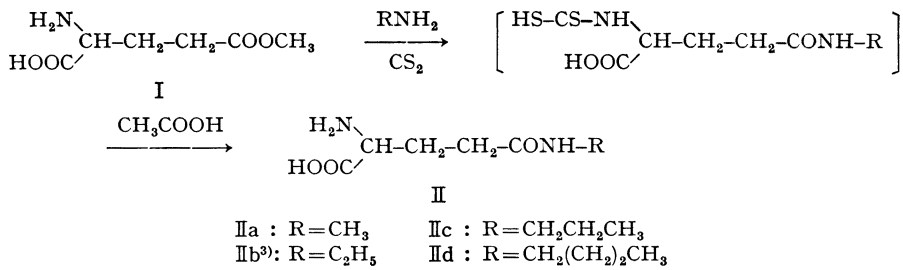


Chart 1

Syntheses of DL-N-alkylglutamines were accomplished by the methods of King, *et al.*[4] and Friedman, *et al.*[4] with some modifications (Chart 2).

1) Part I: R. Kimura and T. Murata, *Chem. Pharm. Bull.* (Tokyo), **19**, 1257 (1971).
2) Location: *2-1, Oshika-2-chome, Shizuoka.*
3) T. Furuyama, T. Yamashita and S. Senoh, *Bull. Chem. Soc. Japan*, **37**, 1078 (1964).
4) F.E. King and D.A.A. Kidd, *J. Chem. Soc.*, **1949**, 3315; O.M. Friedmann and R. Chatterji, *J. Am. Chem. Soc.*, **81**, 3750 (1959).

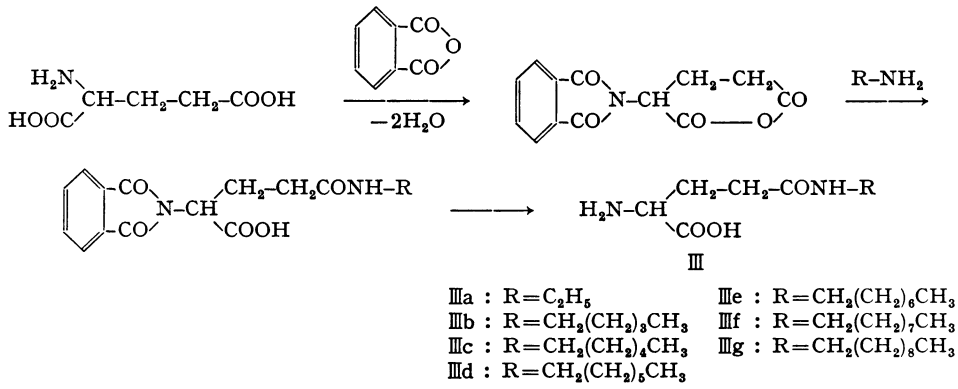


Chart 2

DL-N-Alkyl-2-aminoadipamic acids were prepared by the actions of alkylamines upon DL-2-piperidone-6-carboxylic acid (IV) which was obtained from DL-2-aminoadipic acid by heating (Chart 3).

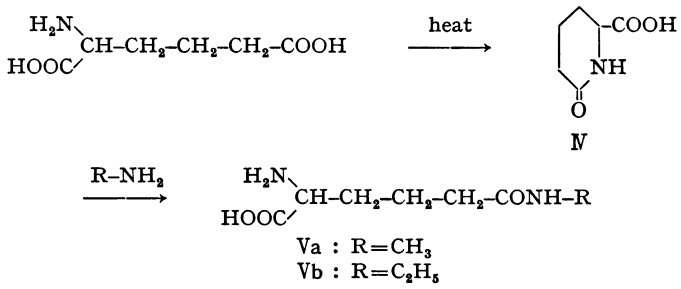


Chart 3

It was unsuccessful to synthesize DL-2-aminoadipamic acid by the above-described method, and ammonium DL-2-piperidone-6-carboxylate was obtained. Akabori and his associate[5] have described that they could not synthesize L-GluNH_2 by the same method but obtained ammonium L-2-pyrrolidone-5-carboxylate. And they were successful in synthesizing L-GluNH_2 by the reduction of L-glutamic acid-5-hydrazide obtained from I. We were able to apply successfully the method of Akabori, *et al.*[5] to the synthesis of DL-2-aminoadipamic acid (Chart 4).

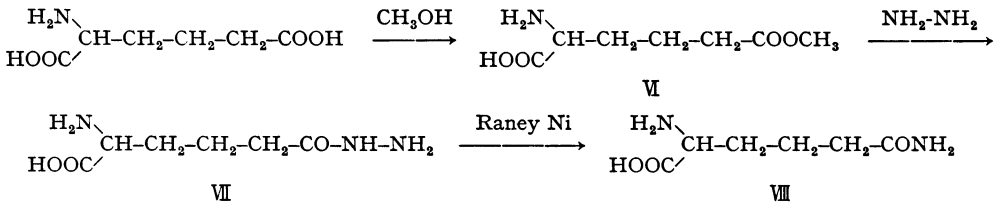


Chart 4

L-N-Alkylasparagines were synthesized by the actions of alkylamines on 4-ethyl L-aspartate[6] (Chart 5).

5) S. Akabori and K. Narita, *Nippon Kagaku Zasshi*, **74**, 829 (1953).
6) T. Hashizume, *Nippon Nogeikagaku Kaishi*, **25**, 127 (1951).

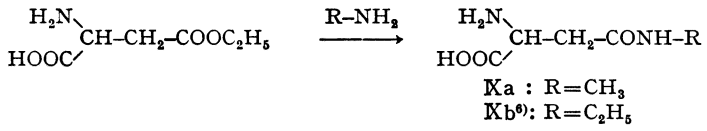
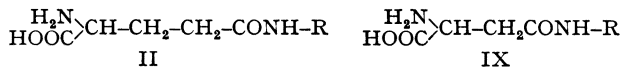


Chart 5

The compounds thus synthesized are listed in Tables I and II.[7] The L-GluNH$_2$ and DL-GluNH$_2$ used in this study were purchased from Wako Pure Chemical Industries and Tokyo Kasei Kogyo Co., respectively.

TABLE I. List of L-Compounds used for the Pharmacological Test[7]



No.	R	mp(decomp.) (°C)	Formula	Analysis (N%) Calcd.	Found	$[\alpha]^t_{5896Å}(H_2O)$	t
IIa	CH_3	201	$C_6H_{12}O_3N_2$	17.49	17.38	+8.3°	16
IIb	C_2H_5[3]	215	$C_7H_{14}O_3N_2$	16.08	15.89	+8.2°	16
IIc	$CH_2CH_2CH_3$	223	$C_8H_{16}O_3N_2$	14.88	14.93	+6.6°	15
IId	$CH_2(CH_2)_2CH_3$	223.5	$C_9H_{18}O_3N_2$	13.85	13.82	+7.4°	16
IXa	CH_3	252	$C_5H_{10}O_3N_2$	19.17	19.15	−3.3°	16
IXb	C_2H_5[6]	258	$C_6H_{12}O_3N_2$	17.49	17.60	−5.1°	15

TABLE II. List of DL-Compounds used for the Pharmacological Test[7]

No.	R	mp(decomp.) (°C)	Formula	Analysis (N%) Calcd.	Found
IIIa	C_2H_5	219	$C_7H_{14}O_3N_2$	16.08	16.03
IIIb	$CH_2(CH_2)_3CH_3$	227	$C_{10}H_{20}O_3N_2$	12.94	13.01
IIIc	$CH_2(CH_2)_4CH_3$	224	$C_{11}H_{22}O_3N_2$	12.17	12.20
IIId	$CH_2(CH_2)_5CH_3$	224.5	$C_{12}H_{24}O_3N_2$	11.47	11.58
IIIe	$CH_2(CH_2)_6CH_3$	225	$C_{13}H_{26}O_3N_2$	10.84	10.84
IIIf	$CH_2(CH_2)_7CH_3$	220	$C_{14}H_{28}O_3N_2$	10.28	10.01
IIIg	$CH_2(CH_2)_8CH_3$	220	$C_{15}H_{30}O_3N_2$	9.78	9.51
Va	CH_3	216.5	$C_7H_{14}O_3N_2$	16.08	15.92
Vb	C_2H_5	225.5	$C_8H_{16}O_3N_2$	14.88	14.87
VIII	H	191	$C_6H_{12}O_3N_2$	17.49	17.36

Experimental

Synthesis

L-N-Alkylglutamine (II)——Under cooling in ice, 2.5 ml of carbon disulfide and 30 ml of dry alkylamine (when methylamine was used, a solution of 35 ml methylamine in 20 ml absolute methanol should be employed) were added to 5.0 g of I, and the mixture in a tightly stoppered flask was shaken till complete dissolution. The resulting transparent solution was allowed to stand at room temperature for 10 to 18 days, followed by elimination of excess alkylamine and carbon disulfide by evaporation under reduced pressure. Thirty milliliters of 5 N acetic acid was then added and dissolved to obtain a homogeneous solution which

7) All melting points are uncorrected. Optical rotations were determined with Nihon-Bunko, Model ORD/UV-5.

was further processed for elimination under reduced pressure. When ethanol was added to the resultant solution, the alkylamide was formed. The alkylamide was recrystalized from water–ethanol. The yields were ranged from 24.4 to 26.2%.

DL-N-Alkylglutamine (III)——N-Phthaloyl-DL-glutamic anhydride was synthesized according to King, *et al.*,[4] and 0.02 moles of the anhydride was dissolved into 30 ml of dioxane. Then 0.021 moles of alkylamine was added to the dioxane solution and the mixture was refluxed for 2.5 hours.

(In case of adding ethylamine, 15 ml of 40% ethylamine dioxane solution was dropped into the N-phthaloyl-DL-glutamic anhydride dioxane solution under cooling. A solid matter was formed during the process and the mixture was allowed to stand overnight. After removing the excess alkylamine and dioxane by distillation under reduced pressure, the residue was dried on sulfuric acid through night.) The mixture was then dissolved in 20 ml of absolute ethanol and was added with 0.022 moles of hydrazine hydrate (100%). After refluxing the mixture for 2 hours, ethanol was eliminated by distillation. The residue was dissolved in 70% ethanol by heating. III was crystallized from the solution by adding acetone, and was recrystallized from 70% ethanol and a small amount of acetone. The yield was not less than 87%. In case on ethylamide, after elimination of ethanol by evaporation under reduced pressure 30 ml of water was added and the mixture was allowed to boil for a few minutes. The mixture was then cooled and filtrated. About 150 ml of ethanol was added to the filtrate thus obtained in order to crystallize DL-N-ethylglutamine, and the product was recrystallized from 80% ethanol, in a yield of 63%.

DL-2-Piperidone-6-carboxylic Acid (IV)——DL-2-Aminoadipic acid, 1.7 g, was melt and the heating was continued on until complete subsidence in bubbling, followed by cooling and recrystallization from ethanol. Yield: 1.3 g (84%), mp 176°.

DL-N-Alkyl-2-aminoadipamic Acid (V)——Compound IV, 0.8 g, was placed along with 7 ml of dried alkylamine (in case of methylamine, a solution of 10 ml of dried methylamine in 10 ml absolute methanol) in a tight-stoppered flask and the mixture was shaken until a homogeneous solution was obtained. The solution was allowed to stand at room temperature for 12 days. Alkylamine was then eliminated by evaporation under reduced pressure. The residue was dissolved in a small amount of ethanol, and insoluble matters were collected. The resulting product was recrystallized from water–ethanol mixture. The yield was 33%.

6-Methyl DL-2-aminoadipate (VI)——DL-2-Aminoadipic acid, 4.0 g, was suspended in absolute methanol and dried hydrogen chloride was passed through the suspension for dissolution. Then the solvent was eliminated by evaporation under reduced pressure, and the remainder was allowed to dry overnight on a sodium hydroxide *in vacuo*. The methyl ester thus obtained was dissolved in 80 ml of ethanol, and the solution was neutralized with pyridine. The resultant crystalline solids were collected by filtration and recrystallized from ethanol-water. The yield was 3.0 g (69%), mp 174° (decomp.). *Anal.* Calcd. for $C_7H_{13}O_4N$: C, 47.99; H, 7.48; N, 8.00. Found: C, 48.25; H, 7.46; N, 7.93.

DL-2-Aminoadipic Acid 6-Hydrazide (VII)——To a suspension of 2.8 g of VI in 20 ml of ethanol, 2.1 g of hydrazine hydrate (100%) was added. When the mixture was stirred and heated, crystalline hydrazide was formed rapidly. The mixture was cooled and filtered, and the hydrazide was recrystallized from ethanol–water mixture. The yield was 1.9 g (66.7%). Further recrystallization from ethanol–water yielded a product, mp 206° (decomp.). *Anal.* Calcd. for $C_6H_{13}O_3N_3$: C, 41.13; H, 7.47; N, 23.99. Found: C, 41.44; H, 7.49; N, 23.77.

DL-2-Aminoadipamic Acid (VIII)——To a suspension of 1.5 g of compound VII in 50% ethanol, Raney Ni (W-5)[8] which had been prepared from 8 g of Ni–Al (Ni, 48%) was added, followed by reflux of the mixture until it became to give a negative Tollens' test (about 30 minutes). After cooling, the mixture was filtered and the catalyst was washed out with hot water. The filtrate and the washings were combined, and a small amount of dimethylglyoxime was added to the combined solution. Then the solution was filtrated and the resulting sediments were washed with warm water. The filtrate and the washings were combined and decolorized with activated charcoal, followed by condensation to a volume of about 15 ml. When the condensate was diluted with ethanol to a volume of 50 ml approx. VIII was crystallized. The yield was 0.9 g (64.9%). Twice recrystallization from ethanol–water mixture led to formation of the compound, mp 191° (decomp.). *Anal.* Calcd. for $C_6H_{12}O_3N_2$: C, 44.99; H, 7.55; N, 17.49. Found: C, 45.16; H, 7.62; N, 17.36.

L-N-Methylasparagine (IXa)——To a solution of 2.0 g of 4-ethyl L-aspartate in 10 ml of absolute ethanol in a tight-stoppered flask, 7 g of dried methylamine in 10 ml absolute ethanol was added under cooling in ice. The mixture was allowed to stand at room temperature for 20 days. Then the excess methylamine and ethanol were eliminated by evaporation under reduced pressure, followed by removal of insoluble matters after addition of 25 ml warm ethanol to the remnant. By subsequent elimination of ethanol by evaporation under reduced pressure, IXa was obtained. Recrystallization of the product from water–ethanol led to the formation of flake crystals, mp 252° (decomp.). The yield was 0.6 g (32%).

8) H. Adkins and H.R. Billica, *J. Am. Chem. Soc.*, **70**, 695 (1948).

Pharmacological Study

Animals——Male mice (ddY strain), weighing from 18 to 20 g and male rats (Wistar strain) weighing approximately 200 g were used.

Inhibition of Caffeine-Induced Lethal Convulsion——Groups of mice, 10 to a group, which had been starved for 16 hours were given with a solution of II or III in water or with suspensions of alkylamides higher than amylamide, being sparingly soluble in water, in 0.5% tragacanth solution in doses of 2.5, 5.0 and 10.0 mmoles/kg by intraperitoneal route. Fifteen minutes after the injection of the alkylamides, an aqueous solution of caffeine was administered in a dose of 270 mg/kg by the same route. Control group was given saline instead of the amides.

Measurement of Oxygen Consumption by Cerebrocortical Slices——Slices of the cerebral cortex, each weighing about 90 mg, were prepared from the brains of rats by the usual method, and oxygen consumptions by the tissues were measured with Warburg manometric apparatus. As an incubation medium, Krebs–Ringer phosphate buffer, pH 7.4,[9] was used with or without 100 mM of potassium chloride. The concentration of test compound used was 10^{-4}M, and 10 mM glucose was added to the medium as the substrate. A cylindrical filter paper impregnated with 0.1 ml of 20% potassium hydroxide was placed in the center well.

Result

Inhibition of Caffeine-Induced Lethal Convulsions

Attempts were made to determine whether and, if any, to what extent the increase in number of carbon atoms of the alkyl group of amide at position 5 of L- or DL-glutamic acid might affect the anticonvulsant effect.

Table III. Effect of L-N-Alkylglutamines on the Mortality Rate of Caffeine in Mice

Alkylamide	Response[a]			
	Control	2.5	5.0	10.0
	10/10			
-NH-H		9/10	6/10	2/10
$-CH_3$		8/10	7/10	—
$-C_2H_5$		7/10	4/10	1/10
$-CH_2CH_2CH_3$		—	3/10	—
$-CH_2(CH_2)_2CH_3$		—	5/10	—

Alkylamide solution was injected intraperitoneally 15 min before intraperitoneal injection of caffeine (270 mg/kg).
a) dose (mmoles/kg)

Table IV. Effect of DL-N-Alkylglutamines on Caffeine–Induced Convulsion in Mice

Alkylamide	Response[a]	
	Control	Amide
	10(10)/10	
-NH-H		4(4)/10
$-C_2H_5$		5(5)/10
$-CH_2(CH_2)_3CH_3$[b]		5(9)/10
$-CH_2(CH_2)_4CH_3$[b]		8(9)/10
$-CH_2(CH_2)_5CH_3$[b]		2(10)/10
$-CH_2(CH_2)_6CH_3$[b]		3(5)/10
$-CH_2(CH_2)_7CH_3$[b]		4(6)/10
$-CH_2(CH_2)_8CH_3$[b]		6(9)/10

Alkylamide solution (5.0 mmoles/kg) was injected intraperitoneally 15 min before intraperitoneal administration of caffeine (270 mg/kg).
a) No. of the dead after the tonic convulsion within 3 hr and No. of the dead within 24 hr (figures in parentheses)
b) suspended in 0.5 % tragacanth solution

9) H.F. DeLuca and P.P Cohen, "Manometric Techniques," 4th ed., ed. by W.W. Umbreit, R.H. Burris and J.F. Stauffer, Burgess, Minneapolis, 1964, p. 132.

As can be seen from Tables III and IV, all the mice in the control group were promptly succumbed to tonic convulsion developing within 45 minutes after intraperitoneal injection of 270 mg/kg of caffeine. By contrast, a substantial reduction in mortality was evident in the groups of mice pretreated with alkylamides. There existed a tendency for the anticonvulsant effect to be augmented with the increase in number of carbon atoms of alkyl group, the maximum effect being seen with its alkyl group possessing seven to eight carbon atoms. Meanwhile, DL-alkylamides with C_5 to C_{10} alkyl radicals were seemed to be toxic, and deaths without convulsion were frequent within 24 hours post administration in the groups of animals receiving these higher alkylamides. Therefore, test was made by comparing number of animals which showed convulsion within 3 hours following injection of caffeine (Table IV). No significant differences were obtained in the response of mice between L-compounds and DL-compounds.

Influence on the Oxygen Consumption by Cerebral Cortex

None of the ω-alkylamides of L-glutamic acid, L-aspartic acid and DL-2-aminoadipic acid was found to be capable of increasing or decreasing the oxygen consumption by slices of rat cerebral cortex (Table V) either with or without potassium effect.

TABLE V. Effect of ω-Alkylamides of L-Glutamic Acid, L-Aspartic Acid and DL-2-Aminoadipic Acid on the Respiration by Rat Brain Cortex Slices

Compound (0.1 mM)	No.[a]	Qo_2[b] Control	Qo_2[b] Amide added	Effect (%)
A) KCl: 5 mM				
L-Asparagine	6	2014±163	2026± 74	+0.6
L-N-Methylasparagine	7	2028±144	2034±132	+0.3
L-N-Ethylasparagine	4	2164±136	2316±362	+7.0
L-Glutamine	5	2138± 59	2080±183	−2.7
L-N-Methylglutamine	7	2177±172	2060±107	−5.4
L-N-Ethylglutamine	8	2075±116	2096±116	+1.0
DL-2-Aminoadipamic acid	5	1924±141	1915±127	−0.5
DL-N-Methyl-2-aminoadipamic acid	5	2148±199	2092± 69	−2.6
DL-N-Ethyl-2-aminoadipamic acid	5	2148±199	2229±137	+3.8
B) KCl: 105 mM				
L-Asparagine	6	2477±190	2250± 72	−9.1
L-N-Methylasparagine	7	2565±153	2411±145	−6.0
L-N-Ethylasparagine	5	2628±291	2577±325	−1.9
L-Glutamine	5	2651±148	2583±197	−2.6
L-N-Methylglutamine	7	2548±190	2458±142	−3.5
L-N-Ethylglutamine	7	2658±146	2644±181	−0.5
DL-2-Aminoadipamic acid	5	2713±181	2657±165	−2.1
DL-N-Methyl-2-aminoadipamic acid	4	2708± 92	2501±105	−7.6
DL-N-Ethyl-2-aminoadipamic acid	4	2708± 92	2534±117	−6.4

a) number of experiments
b) Qo_2 is expressed as the mean ±S.D. (μl/g wet weight/hr).

Discussion

In a previous study[1] L-GluNHEt was found to be more effective than L-GluNH_2 in suppressing the lethal convulsion induced by caffeine. In relation to the finding the present study was carried out to make assessment of a series of newly synthesized alkylamides with alkyl groups of higher members for their pharmacological activity with particular reference to anticonvulsant effect against caffeine. As a result, maximum anticonvulsive efficacy

was observed with alkylamides possessing C_7 to C_8 alkyl groups (Tables III and IV). Possible explanation may include the increased passability through the blood brain barrier due to increase in fat-solubility of the compounds with greater alkyl radicals, and, as has been described by Hawkins, *et al.*,[10] the reduction in the rate of metabolism prior to uptake by the brain tissue. With the increase in number of carbon atoms, however, toxicity of alkylamides eventually became augmented; compounds with alkyl groups possessing five to seven carbon atoms displayed the highest toxity. As the disturbance in the energy production system of the brain tissue has been implicated, among others, in the genesis of convulsion,[11] *in vitro* experiments were carried out to determine whether a series of alkylamides of acidic amino acids might affect the respiration by the cerebral cortex by using rat brain slices. The results indicated that none of the alkylamides tested were capable of increasing or diminishing the cerebrocortical oxygen consumption (Table V).

Acknowledgement The authors are deeply indebted to the members of the analytical room of this college for elemental analyses and measurement for optical rotations.

10) J.E. Hawkins, Jr. and L.H. Sarett, *Clin. Chim. Acta*, **2**, 481 (1957).
11) H. Naruse and T. Kariya, "Biochemistry of Brain," 1st ed., ed. by Y. Tsukada, Igakushoin, Tokyo, 1964, p. 600.