Notes

Chem. Pharm. Bull. 26(11)3546—3551(1978)

UDC 547.466.1.04.09 : 615.37.011.5.015.11

Study on Influence of Synthetic Glucagon Fragment on Lymphocyte Transformation. I[1]

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(Received November 10, 1977)

The tetradecapeptide corresponding to position 1 to 14 of glucagon was synthesized by a conventional method. Though the suppression test of this peptide was tested against PHA-induced lymphocyte transformation, any reproducible and significant results were not obtained. In this finding, the effect on immunodeficiency of chronic renal failure was not because of glucagon fragment (1—14).

Keywords——hyperglucagonemia; middle molecular substance; lymphocyte transformation; chronic renal failure; uremia

MMS accumulated in uremia was shown to influence to Hb synthesis,[3] neuropathy,[4] suppression of PHA-induced lymphocyte transformation[5] and inhibition of erythrocyte glycolysis.[6] MMS is probably peptide and has molecular weight 500—1600.[4] On the other hand, carbohydrate intolerance was found to patients with uremia.[7] Recentry, Bilbrey *et al.*[8] obserbed increased levels of circulating glucagon in chronic renal failure. The hyperglucagonemia of chronic renal failure is largely due to an increase in peptide of approximately m.w. 9000 consistent with proglucagon, although the 3500 component is also considerably elevated to threefold[9] greater in uremic patients than controls. It was found that hyperglucagonemia in uremia is primarily a result of decreased catabolism rather than hypersecretion of this hormone.[10] Glucagon for the symptomatology and metabolic aberrations in uremia are still insufficiently known.[8] The peptide nature of the MMS isolated by Bricker *et al.*[11] might suggest that some of these middle molecular fractions contain peptide hormones

1) The amino acid residues except glycine are of the L-configuration. The abbrebiations used to denote amino acid derivatives and peptides are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochem. Biophys. Acta*, **263**, 205 (1972). Other abbreviations: DMF=dimethylformamide, Et_3N=triethylamine, TFA=trifluoroacetic acid, DCC=dicyclohexylcarbodiimide, HOBt=N-hydroxybenztriazole, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, DCU=dicyclohexyl urea, MMS=middle molecular substance, Tos=*p*-toluenesulfonic aicd, MSA=methanesulfonic acid, PHA=phytohaemaglutinin, Hb=hemoglobin.

2) Location: *Aramaki-aza-sanbonmatsu 9-1, Sendai, 980, Japan.*

3) G. Goubead, H.W. Leber, H.H. Schott and G. Schütterle, *Kidney International*, **10**, 195 (1976).

4) P. Fürst, J. Bergström, A. Gordon, E. Johnsson and L. Zimmerman, *Kidney International*, **7**, S-272 (1975).

5) Z. Hanicki, T. Cichoki, M. Sarnecka-Keller, A. Alein and K. Komorowska, *Nephron*, **17**, 73 (1976).

6) M. Gajdos and R. Dzúrick, *Int. Urol. Nephrol.*, **5**, 331 (1973).

7) F.B. Westernelt and G.E. Schreiner, *Ann. Intern. Med.*, **57**, 266 (1962).

8) G.L. Bilbrey, G.G. Faloona, M.G. White, C. Atkins, A.R. Hull and J.D. Knochel, *Ann. Intern. Med.*, **82**, 525 (1975).

9) S.F. Kuku, J.B. Jaspan, D.S. Emmanouel, A. Zeidler, A.I. Katz and A.H. Rubenstein, *J. Clin. Invest.*, **58**, 743 (1976).

10) R.S. Sherwin, C. Bastl, F.O. Finkelstein, M. Fisher, H. Black, R. Hendler and P. Felig, *J. Clin. Invest.*, **57**, 722 (1976).

11) N.S. Bricker, *New Eng. J. Med.*, **286**, 1093 (1972).

or products of hormone degradation. There is a posibility of the appearance of the other glucagon fragments as MMS resulting from unknown enzymatic degradation of glucagon in uremic patients. It is obvious that the physiologically active glucagon fragments are accumulated due to inhibition of excretion cause chronic renal failure. The authers, have synthesized glucagon fragment (1—14) by conventional method to examine suppression of PHA-induced lymphocyte transformation.[5,12] The inhibition activity of the glucagon fragment (1—14) was tested against lymphocyte stimulation by PHA. The result was summerized in Table I. This peptide fragment of glucagon exhibited no inhibition activity of lymphocyte stimulation by PHA.

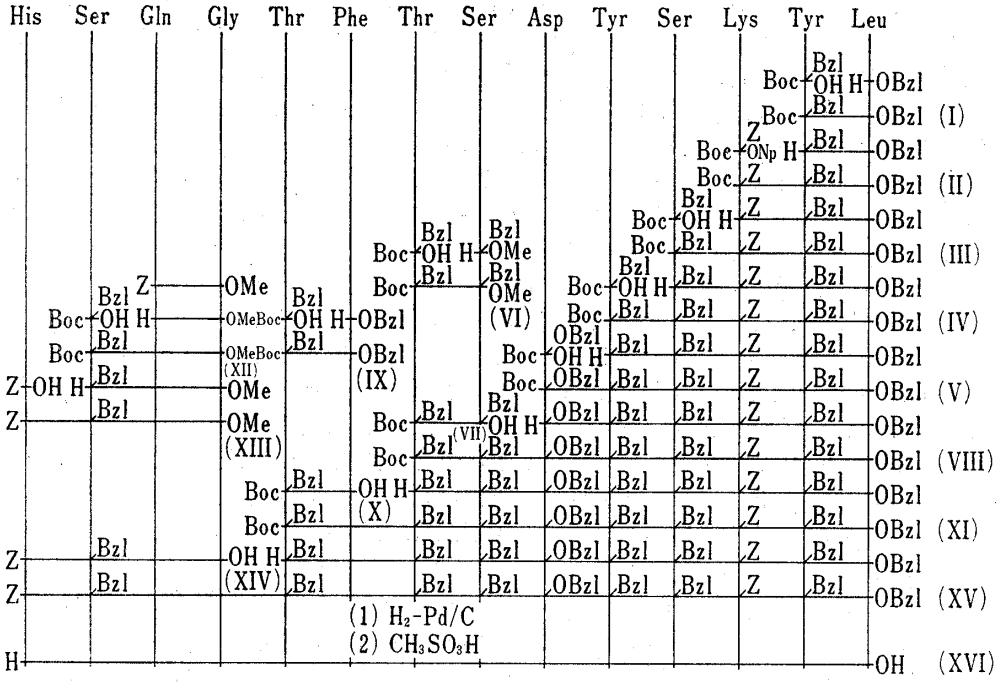

Fig. 1. Synthetic Scheme of the Glucagon Fragment (1—14)

Table I. Influence of Glucagon Fragment (1—14) on Stimulation of Normal Lymphocyte by PHA

Concentration of glucagon fragment (1—14) mg/ml	^{3}H-Thymidine incorporation cpm	Concentration of[a] V.B_{12} (m.w. 1355) mg/ml	^{3}H-Thymidine incorporation cpm
0.002	42070	0.0012	41710
0.020	42580	0.0120	43930
0.200	44300	0.1200	46630
2.000	41940	1.2000	39780

a) Control.

In this paper, we described in detail the synthesis of glucagon fragment (1—14) (Fig. 1). As shown in Fig. 1, the main strategy for the synthesis of the tetradecapeptide is as follows: H-Leu-OBzl Tos was condensed with Boc-Tyr(Bzl)-OH by the DCC-HOBt method[13] to yield Boc-Tyr(Bzl)-Leu-OBzl (I). I was treated with TFA in the presence of anisole and the result-

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13) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

ing dipeptide ester was condensed with Boc-Lys(Z)-ONp[14] to yield Boc-Lys(Z)-Tyr(Bzl)-Leu-OBzl (II). After removal of the Boc group of II with TFA, the resulting tripeptide ester was condensed with Boc-Ser(Bzl)-OH[15] as described in I to yield Boc-Ser(Bzl)-Lys(Z)-Tyr-(Bzl)-Leu-OBzl (III). After removal of the Boc group of III with TFA, the resulting tetrapeptide ester was condensed with Boc-Tyr(Bzl)-OH as described in I to yield Boc-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl (IV). After removal of the Boc group of IV with TFA, the resulting pentapeptide ester was condensed with Boc-Asp(OBzl)-OH as described in I to yield Boc-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl (V). H-Ser(Bzl)-OMe HCl was condensed with Boc-Thr(Bzl)-OH as described in I to yield Boc-Thr(Bzl)-Ser(Bzl)-OMe (VI). Saponification of VI with 1 N NaOH afforded Boc-Thr(Bzl)-Ser(Bzl)-OH (VII). After removal of the Boc group of V with TFA, the resulting hexapeptide ester was condensed with VII by the DCC-HONB method[16] to yield Boc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser-(Bzl)-Lys(Z)-Leu-OBzl (VIII). H-Phe-OBzl Tos was condensed with Boc-Thr(Bzl)-OH as described I to yield Boc-Thr(Bzl)-Phe-OBzl (IX). Saponification of IX with 1 N NaOH afforded BocThr(Bzl)-Phe-OH (X). After removal of the Boc group of VIII with TFA, the resulting ocatpeptide ester was condensed with X as described in VIII to yield Boc-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl (XI). After removal of the Z group of Z-Gln-Gly-OMe[17] by the catalytic hydrogenation, the resulting dipeptide ester was condensed with Boc-Ser(Bzl)-OH as described in I to yield Boc-Ser-(Bzl)Gln-Gly-OMe (XII). After removal of the Boc group of XII with TFA, the resulting tripeptide ester was condensed with Z-His-OH as described I to yield Z-His-Ser(Bzl)-Gln-Gly-OMe (XIII). Saponification of XIII with 1 N NaOH afforded Z-His-Ser(Bzl)-Gln-Gly-OH (XIV). After removal of the Boc group of XI with TFA, the resulting decapeptide ester was condensed with XIV as described in I to yield Z-His-Ser(Bzl)-Gln-Gly-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Try(Bzl)-Leu-OBzl (XV). The protected tetradecapeptide ester was treated with AcOH-MeOH to remove the contaminating dicyclohexylamidino derivative[18] to yield pure XV. The fully protected tetradecapeptide was hydrogenated over 5% palladium on the carbon in aqueous AcOH for 36 hr. Paper chromatography of hydrogenated product gave about 30% of minor spot by ninhydrin and pauly reagent being probably due to partially protected peptide. The hydrogenated product was treated with anhyd. MSA in the presence of anisole.[19] The deblocked tetradecapeptide was purified through Sephadex G-15 column to obtain tetradecapeptide (XVI). The tetradecapeptide XVI thus obtained was found to be homogeneous from result of paper chromatography using two different solvent systems. Ratios of amino acids in the acid hydrolysates and AP-M digest[20] of peptide were agreed well with those of theory.

Experimental

Melting points are uncorrected. For paper chromatography, the protected peptides were deblocked with TFA unless otherwise mentioned and resulting trifluoroacetate were chromatographed on a filter paper Toyo Roshi No. 51, at room temperature. Rf^1 value refer to the Partridge system[21] and Rf^2 value to the system of BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24).[22] The amino acid composition of the acid hydroly-

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sate and AP-M digest determined with JEOL-JLC-8AH amino acid analyzer according to the directions given by Moore *et al.*[23]

Boc-Tyr(Bzl)-Leu-OBzl (I)——To a solution of Boc-Tyr(Bzl)-OH (4.1 g), H-Leu-OBzl Tos (3.9 g) and Et_3N (1.5 ml) in DMF (30 ml) were added HOBt (1.5 g) and DCC (2.3 g) with stirring at 0°. The mixture was stirred at 0° for 2 hr and additional 12 hr at 4°. The reaction mixture was filtered off to remove the formed DCU, and the filtrate was diluted with EtOAc. The EtOAc-extracts were washed with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O, dried over anhyd. $MgSO_4$ and evaporated to dryness in vacuum. The residue was precipitated from EtOAc and petroleum ether. Yield 4.8 g (84%), mp 84—86°, $[\alpha]_D^{23}$ −23.6° (c=0.4, DMF), *Anal.* Calcd. for $C_{34}H_{42}N_2O_6$: C, 71.05; H, 7.37; N, 4.88. Found: C, 71.43; H, 7.95; N, 4.95. The deblocked peptide ester: Rf^1 0.92, Rf^2 0.97, single ninhydrin positive spot.

Boc-Lys(Z)-Tyr(Bzl)-Leu-OBzl (II)——The protected dipeptide ester (I) (2.9 g) was dissolved in TFA (6 ml) and the solution was kept at room temperature for 30 min. The mixture was evaporated in vacuum and dried over KOH in vacuum. To a solution of this product in DMF (20 ml) was added Boc-Lys(Z)-ONp (2.1 g),[14] followed by the addition of Et_3N to keep the solution slightly alkaline. After 24 hr at room temperature, the reaction mixture was diluted with 1 N NH_4OH (15 ml), stirred for 1 hr, and then extracted with EtOAc (120 ml). The EtOAc solution was washed successively with 1 N NH_4OH, H_2O, 1 N citric acid and H_2O and dried over anhyd. $MgSO_4$. The solution was concentrated to a small volume and petroleum ether was added to the residue. The precipitate was reprecipitated from DMF and 1 N NH_4OH. yield 3.4 g (80%), mp 103—110°, $[\alpha]_D^{23}$ +27.2° (c=0.3, DMF), *Anal.* Calcd. for $C_{48}H_{60}N_4O_9$: C, 68.89; H, 7.23; N, 6.70. Found: C, 68.90; H, 7.28; N, 7.14. The deblocked peptide ester: Rf^1 0.87, Rf^2 0.94, single ninhydrin positive spot.

Boc-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl Monohydrate (III)——The protected tripeptide ester (II) (2.8 g) was treated with TFA as described above. The protected tetrapeptide ester (III) was prepared from this deblocked tripeptide ester of II, Boc-Ser(Bzl)-OH (1.1 g), DCC (0.8 g) and HOBt (0.5 g) essentialy in the same manner as described in the preparation of I. The product was reprecipitated from ether and petroleum ether. Yield 3.1 g (89%), mp 87—90°, $[\alpha]_D^{23}$ −23.7° (c=0.4, DMF), *Anal.* Calcd. for $C_{58}H_{71}N_5O_{11}\cdot H_2O$: C, 67.49; H, 7.13; N, 6.79. Found: C, 67.57; H, 6.73; N, 6.83. The deblocked peptide ester: Rf^1 0.88, Rf^2 0.93, single ninhydrin positive spot.

Boc-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl Monohydrate (IV)——The protected tetrapeptide ester (III) (2.0 g) was treated with TFA as described above. The protected pentapeptide ester (IV) was prepared from this deblocked tetrapeptide ester of III, Boc-Tyr(Bzl)-OH (0.8 g), DCC (0.5 g) and HOBt (0.3 g) essentially in the same manner as described in the preparation of I. The product was reprecipitated from tetrahydrofuran and petroleum ether. Yield 1.6 g (64%), mp 75—86°, $[\alpha]_D^{23}$ −39.6° (c=0.2, DMF), *Anal.* Calcd. for $C_{74}H_{86}N_6O_{13}H_2O$: C, 69.14; H, 6.90; N, 6.54. Found: C, 69.47; H, 6.67; N, 6.57. The deblocked peptide ester: Rf^1 0.89, Rf^2 0.94, single ninhydrin positive spot.

Boc-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl (V)——The protected pentapeptide ester (IV) (1.3 g) was treated with TFA as described above. The protected hexapeptide ester (V) was prepared from this deblocked tetrapeptide ester of IV, Boc-Asp(OBzl)-OH (0.40 g), DCC (0.23 g) and HOBt (0.15 g) essentially in the same manner as described in the preparation of I. The product was reprecipitated from tetrahydrofuran and petroleum ether. Yield 1.10 g (73%), mp 125—136°, $[\alpha]_D^{23}$ +28.6° (c=0.4, DMF), *Anal.* Calcd. for $C_{85}H_{97}N_7O_{16}$: C, 70.12; H, 6.84; N, 6.66. Found: C, 70.08; H, 6.57; H, 6.71. The deblocked peptide ester: Rf^1 0.96, Rf^2 0.94, single ninhydrin positive spot.

Boc-Thr(Bzl)-Ser(Bzl)-OMe (VI)——This protected dipeptide ester (VI) was prepared from H-Ser(Bzl)-OMe HCl (1.2 g), Boc-Thr(Bzl)-OH (1.7 g), DCC (1.1 g) amd HOBt (0.7 g) essentially in the same manner as described in the preparation of I. Yield 1.0 g (40%), mp 117—118°, $[\alpha]_D^{23}$ +30.5° (c=0.3, DMF), *Anal.* Calcd. for $C_{27}H_{36}N_2O_7$: C, 64.78; H, 7.25; N, 5.60. Found: C, 65.21; H, 7.71; N, 5.41. The deblocked peptide ester: Rf^1 0.85, Rf^2 0.90, single ninhydrin positive spot.

Boc-Thr(Bzl)-Ser(Bzl)-OH(VII)——The protected dipeptide ester (VI) (1.0 g) in dioxane (10.0 ml) was saponified with 1 N NaOH (2.2 ml) for 1 hr at room temperature and diluted with H_2O (30 ml). The solution was washed with EtOAc twice and aqueous layer was acidified with 2 M citric acid to congo red and saturated with NaCl. The precipitate was extracted with EtOAc and the solution was washed with H_2O. The EtOAc layer was dried over anhyd. $MgSO_4$ and the solvent was evaporated in vacuum. The residue was reprecipitated from EtOAc and petroleum ether. Yield 0.8 g (83%), $[\alpha]_D^{23}$ +36.6° (c=0.3, DMF), *Anal.* Calcd. for $C_{26}H_{34}N_2O_7$: C, 63.40; H, 6.96; N, 6.91. Found: C, 63.01; H, 7.44; N, 6.46. The deblocked peptide ester: Rf^1 0.65, Rf^2 0.76, single ninhydrin positive spot.

Boc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl Dihydrate (VIII)——The protected hexapeptide ester (V) (589 mg) was treated with TFA as described II. The protected octapeptide ester (VIII) was prepared from this deblocked hexapeptide ester of V, Boc-Thr(Bzl)-Ser(Bzl)-OH(VII) (215 mg), DCC (90 mg) and HONB (60 mg)[16] essentially in the same manner as described in the preparation of I. The product was reprecipitated from MeOH and ether. Yield 450 mg (60%), mp 108—115°, $[\alpha]_D^{23}$

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$-22.3°$ ($c=0.3$, DMF), *Anal.* Calcd. for $C_{106}H_{121}N_9O_{20}\cdot 2HO_2$: C, 67.82; H, 6.71; N, 6.72. Found: C, 67.44; H, 6.85; N, 6.90. The deblocked peptide ester: Rf^1 0.86, Rf^2 0.96, single ninhydrin positive spot.

Boc-Thr(Bzl)-Phe-OBzl (IX)——This protected dipeptide ester (IX) was prepared from H-Phe-OBzl Tos (2.1 g), Boc-Thr(Bzl)-OH (1.6 g), DCC (1.2 g) and HOBt (0.8 g) essentially in the same manner as described in the preparation of I. The product was reprecipitated from EtOAc and ether. Yield 2.0 g (77%), mp 132°, $[\alpha]_D^{23}$ $-14.6°$ ($c=0.4$, DMF), *Anal.* Calcd. for $C_{31}H_{36}N_2O_6$: C, 69.90; H, 6.81; N, 5.26. Found: C, 69.45; H, 6.61; N, 4.98. The deblocked peptide ester: Rf^1 0.89, Rf^2 0.92, sigle ninhydrin positive spot.

Boc-Thr(Bzl)-Phe-OH (X)——The compound was prepared from IX (1.3 g) essentially in the same manner as described in the preparation of VII. Yield 0.9 g (82%), mp 107°, $[\alpha]_D^{23}$ $-14.0°$ ($c=0.3$, DMF), *Anal.* Calcd. for $C_{25}H_{32}N_2O_6$: C, 65.80; H, 7.02; N, 6.14. Found: C, 65.31; H, 7.45; N, 5.88. The deblocked peptide: Rf^1 0.58, Rf^2 0.60, single ninhydrin positive spot.

Boc-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl (IX)——The protected octapeptide ester(VIII) (460 mg) was treated with TFA as described II. The protected decapeptide ester (XI) was prepared from this deblocked octapeptide ester of VIII, Boc-Thr(Bzl)-Phe-OH (X) (136 mg), DCC (56 mg) and HONB (45 mg)[16] essentially in the same manner as described in the preparation of I. The product was reprecipitated from EtOAc and ether. Yield 378 mg (69%), mp 90—94°, $[\alpha]_D^{23}$ $-20.1°$ ($c=0.3$, DMF), *Anal.* Calcd. for $C_{126}H_{143}O_{28}N_{11}$: C, 69.43; H, 6.61; N, 7.07. Found: C, 68.94; H, 6.97; N, 7.50. The deblocked peptide ester: Rf^1 0.96, Rf^2 0.97, single ninhydrin positive spot.

Boc-Ser(Bzl)-Gln-Gly-OMe Monohydrate (XII)——Z-Gln-Gly-OMe[17] (2.9 g) was hydrogenated in MeOH (25 ml), AcOH (1 ml) and H_2O (4 ml) over 5% palladium on the carbon in the usual manner until the evolution of CO_2 ceased. The catalist was removed by filtration and the filtrate was evaporated in vacuum. The protected tripeptide ester (XII) was prepared from this deblocked dipeptide ester, Boc-Ser(Bzl)-OH (2.8 g), DCC (1.9 g) and HOBt (1.2 g) essentially in the same manner as described in the preparation of I. Yield 3.0 g (71%), mp 73°, $[\alpha]_D^{23}$ $-15.2°$ ($c=0.2$, DMF), *Anal.* Calcd. for $C_{23}H_{35}N_4O_8\cdot H_2O$: C, 53.79; H, 7.26; N, 10.91. Found: C, 57.79; H, 7.27; N, 11.12. The deblocked peptide ester: Rf^1 0.79, Rf^2 0.82, single ninhydrin positive spot.

Z-His-Ser(Bzl)-Gln-Gly-OMe (XIII)——The protected tripeptide ester (XII) (1.000 g) was treated with TFA as described II. The protected tetrapeptide ester (XIII) was prepared from this deblocked tripeptide ester of XII, Z-His-OH (0.637 g), DCC (0.500 g) amd HOBt (0.298 g) essentially in the same manner as described in the preparation of I. This product was then dissolved in MeOH (15 ml)–2 N AcOH (5 ml) and the solution, after heating at 60° for 5 hr to remove the contaminating dicyclohexylamidino derivative,[18] was condensed in vacuum. The residue was precipitated from EtOAc and petroleum ether. Yield 0.700 g (50%), mp 95—104°, $[\alpha]_D^{23}$ $+10.1°$ ($c=0.3$, DMF), *Anal.* Calcd. for $C_{32}H_{40}N_7O_9$: C, 57.65; H, 6.05; N, 14.71. Found: C, 57.21; H, 6.41; N, 14.28. The de-Z peptide ester acetate by catalytic hydrogenation: Rf^1 0.69, Rf^2 0.78, single ninhydrin and pauly positive spot.

Z-His-Ser(Bzl)-Gln-Gly-OH (XIV)——The compound was prepared from XIII (556 mg) essentially in the same manner as described in the preparation of VII. The product was reprecipitated from EtOAc and ether. Yield 450 mg (68%), mp 120—125°, $[\alpha]_D^{23}$ $+12.3°$ ($c=0.3$, DMF), *Anal.* Calcd. for $C_{31}H_{38}N_7O_9$: C, 57.04; H, 5.87; N, 15.02. Found: C, 57.05; H, 6.26; H, 14.78. The de-Z peptide by catalytic hydrogenation: Rf^1 0.30, Rf^2 0.30, single ninhydrin and pauly positive spot.

Z-His-Ser(Bzl)-Gln-Gly-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(Z)-Tyr(Bzl)-Leu-OBzl Monohydrate (XV)——The protected decapeptide ester (XI) (218 mg) was treated with TFA as described II. The protected tetrapeptide ester was prepared from this deblocked decapeptide ester of XI, Z-His-Ser(Bzl)-Gln-Gly-OH (XIV) (71 mg), DCC (23 mg) and HOBt (20 mg) essentially in the same manner as described in the preparation of I. This product was dissolved in MeOH (10 ml)–2 N AcOH (1 ml) and the solution, after heating at 60° for 5 hr to removed the contaminating dicyclohexylamidino derivative,[18] was condensed in vacuum. The residue was reprecipitated from EtOAc and ether. Yield 186 mg (68%), mp 120—137°, $[\alpha]_D^{23}$ $-17.0°$ ($c=0.3$, DMF), *Anal.* Calcd. for $C_{152}H_{171}N_{18}O_{29}\cdot H_2O$: C, 66.82; H, 6.38; N, 9.23. Found: C, 67.31; H, 6.49; N, 9.61.

H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-OH (XVI)——The fully protected tetradecapeptide (XV) (90 mg) was hydrogenated in 2: 1 AcOH and H_2O (15 ml) in the presence of 5% palladium on the carbon for 36 hr. The catalyst removed by the aid of cellite. The solution was evaporated to dryness in vacuum and the residue was dried over KOH in vacuum. The crude hydrogenated tetradecapeptide was treated with MSA (1 ml) in the presence of anisole (0.1 ml) at room temperature for 60 min. To the reaction mixture was added H_2O (10 ml) at 0° and washed well with ether. The aqueous layer was passed through a column (1.8×6 cm) of Amberlite IRA-410 (AcO^-). The eluate and washings were combined and evaporated in vacuum. The residue was dissolved in a small amount of H_2O and the solution was applied to a column (1.8×90 cm) of Sephadex G-15, which was eluted with 0.1 N AcOH. Fractions of 4.3 ml of each were collected at a flow rate of 1 ml/min with an automatic fraction collector and the absorbancy of each fraction was determined at 260 nm and 230 nm. The eluate in tubes No. 56 to 68 containing the tetradecapeptide were pooled, evaporated to dryness in vacuum and lyophilized. Yield 28 mg (52%), mp 168—172° (dec.), $[\alpha]_D^{20}$ $-40.0°$ ($c=0.3$, H_2O), Rf^1 0.14, Rf^2 0.16, single ninhydrin and Pauly positive spot, amino acid ratios in the acid hydrolysate: His 0.89, Ser 2.87, Glu 0.91, Asp 0.90, Gly 0.94, Thr 1.88, Phe 0.83, Tyr 1.96, Lys 0.92,

Leu 1.01, amino acid ratios in the AP-M digest: His 1.01, Ser 2.91, Gln 0.90, Asp 0.87, Gly 1.00, Thr 1.98, Phe 0.91, Tyr 1.98, Lys 0.88, Leu 0.92.

Inhibition Activity of Glucagon Fragment on Lymphocyte Stimulation by PHA——Cells were cultured in 0.2 ml of minimum essential medium in microtiter plates (Falcon # 3040). 0.02 ml (final 1 μg/ml) of PHA is added, either 0.02 ml of PHA-induced lymphocyte transformation testing substance or standard substance (VB_{12}). Triplicate cultures of each combination of 5×10^5 cells per well were incubated at 37° in a humidified atmosphere of 5% CO_2 in air for three days. Twenty-four hours before harvest, 5 μCi of ^{3}H-thymidine was added per culture. The amount of thymidine incorporated into DNA measured in a scintilator. The isotope incorporation was not inhibited in the concentration of 2.0 mg/ml of this peptide.

Acknowledgement We thank Prof. K. Kumagai of Department of Microbiology, Tohoku University School of Dentisitry for suppression test of PHA-induced lymphocyte transformation, and the Central analysis, Room of Pharmaceutical Institute, Tohoku University for elemental analysis.

[Chem. Pharm. Bull. 26(11)3551—3555(1978)]

UDC 615.213.015.42.076.9 : 547.295.09

Anticonvulsant Activity and Effects of Sodium Dipropylacetate on Cerebral 5-Hydroxytryptamine and γ-Aminobutyric Acid in Reserpinized Mice

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(Received December 20, 1977)

The anticonvulsant activity of sodium dipropylacetate (DPA) and its effects on the cerebral 5-hydroxytryptamine (5HT) and γ-aminobutyric acid (GABA) metabolisms in the reserpinized mice have been studied to know the mechanism of action of DPA. The results are as follows;

1. DPA elevated the turnover of the cerebral 5HT and the content of the cerebral 5-hydroxyindoleacetic acid. Phenobartital sodium (PB) showed no effects.
2. DPA elevated the cerebral GABA content in the reserpinized mice.
3. In maximal electroshock seizure, the seizure-protecting activities of DPA and PB were lower in the reserpinized mice than in the normal.
4. In pentylenetetrazole seizure, the seizure-protecting activity of DPA in the reserpinized mice was the same as in the normal. In combined administration, L-dihydroxyphenylalanine decreased the activity of DPA in the reserpinized mice. The activity of PB decreased in the reserpinized mice.

From these results, it was suggested that DPA showed the anticonvulsant activity partially through its effect on the cerebral metabolism of 5HT as well as GABA.

Keywords——sodium dipropylacetate; mouse; reserpine; anticonvulsant activity; brain; GABA; 5HT

The effects of sodium dipropylacetate (DPA), an anticonvulsant, on the metabolisms of γ-aminobutyric acid (GABA) and biogenic amines in the rat brain were previously reported.[2] DPA increased the cerebral concentration of tryptophan, 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) in addition to that of GABA. From these results, the possibility that DPA elevated the cerebral turnover of 5HT was proposed.

There have been many reports that provided the role of cerebral GABA as an inhibitory transmitter and showed an intimate relationship between the GABA metabolism and convul-

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