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.

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

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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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sions,[3] while the relationship between the biogenic amine metabolisms and convulsions have not yet been clarified.[4] In the reserpinized mice, the contents of the cerebral 5HT and catecholamines were decreased, and also the thresholds to provoke the convulsions in the experimental seizures were lowered.[5] Therefore, the study on the effects of a drug in the reserpinized mice will be useful to know the mechanism of its anticonvulsant activity.

In this report, the anticonvulsant activity and the effects of DPA on the cerebral concentrations of 5HT and GABA have been investigated in the reserpinized mice.

Experimental

Animals——Male, dd strain mice, weighing 24±1 g, were used and allowed free access to food and water at all times. Reserpine injection (Apoplon Injection, 1 mg/ml) was purchased from Daiichi Seiyaku Co. Ltd. and was diluted with distilled water. The mice were administered intraperitoneally (*i.p.*) the solution of reserpine (5 mg/kg) in a volume of 0.2 ml/mouse at 1:00 p.m., and after 24 hr of the administration they were subjected to the experiment. DPA or phenobarbital sodium (PB) dissolved in saline was administered *i.p.* in a volume of 0.2 ml/mouse. Each control mouse was injected with 0.2 ml saline.

Anticonvulsant Activity——In case of assay of anti-maximal electroshock (MES) seizure, the mice received electroshock at the stated time after drug administration (Woodbury and Davenport type electroshock apparatus, 2000 V, 50 mA, 0.2 sec). The seizure response was judged from the appearance of the tonic extensor. For assay of anti-pentylenetetrazole (PTZ) seizure, PTZ solution in saline (0.2 ml/mouse) was *i.p.* injected after the administration of drugs. The clonic convulsion appeared within 30 min after PTZ injection was observed. The threshold dose of PTZ provoking the seizure was lower in reserpinized mice (70 mg/kg) than in normal mice (100 mg/kg).

Methods of Analyses——The concentrations of brain 5HT and 5HIAA were determined fluorometrically by the method of Curzon and Green.[6] Three brains were combined as a sample. Tryptophan in the serum was estimated by the method of Denckla and Dewey,[7] and DPA in the serum by the method of Kukino, *et al.*[8] The combined serum was passed through the ultrafiltration membrane (Diaflo-01-T), and the filtrate which contained DPA and tryptophan in free form was obtained. In the case of GABA, the mouse was decapitated and the head was dropped into dry ice-isopentane. The whole brain was dissected out on ice and submitted to analysis by the method of Sandman.[9]

Results

Anticonvulsant Activity (Table I)

The effects of DPA and PB on two model convulsions were tested. The time when the effect of each drug was examined was decided from the results of the time course of the effect in the normal mice[10] (the maximal effective time, DPA *i.p.* 15—20 min, PB *i.p.* 60 min).

In the case of MES, the efficacies of DPA and PB were lower in the reserpinized mice than in the normal. L-Dihydroxyphenylalanine(L-DOPA) and L-5-hydroxytryptophan (L-5HTP) had no anticonvulsant activity in the normal and in the reserpinized mice. And in the case of combined administration of DPA or PB with L-DOPA or L-5HTP, the anticonvulsant activities were not different from the single administration of DPA or PB both in the reserpinized and in the normal.

In the studies of PTZ seizure, the efficacy of DPA was equal both in the reserpinized and in the normal. However, the efficacy of PB was lower in the reserpinized than in the normal.

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L-5HTP had weak anticonvulsant activity in the reserpinized mice. Whereas L-DOPA had no activity both in the reserpinized and in the normal. And in the case of combined administration of DPA and L-DOPA, L-DOPA reduced the efficacy of DPA in the reserpinized mice.

TABLE I. Anticonvulsant Activities of Drugs in Maximal Electroshock and Pentylenetetrazole Seizures

	MES seizure		PTZ seizure	
	Normal	Reserpinized	Normal	Reserpinized
Control	20	20	20	20
DPA 50 mg/kg *i.p.* 20 min	—	—	17	15
DPA 100 mg/kg *i.p.* 20 min	—	—	10	9
DPA 200 mg/kg *i.p.* 20 min	10	20	3	3
DPA 250 mg/kg *i.p.* 20 min	9	18	—	—
DPA 300 mg/kg *i.p.* 20 min	3	17	—	—
DPA 400 mg/kg *i.p.* 20 min	1	3	—	—
PB 8 mg/kg *i.p.* 60 min	—	—	18	19
PB 20 mg/kg *i.p.* 60 min	3	19	8	17
L-DOPA 300 mg/kg *p.o.* 60 min	20	20	20	20
L-5HTP 300 mg/kg *p.o.* 60 min	20	20	20	15
DPA 50 mg/kg+L-DOPA 300 mg/kg	—	—	18	19
DPA 50 mg/kg+L-5HTP 300 mg/kg	—	—	15	12
DPA 200 mg/kg+L-DOPA 300 mg/kg	8	18	—	—
DPA 200 mg/kg+L-5HTP 300 mg/kg	8	17	—	—
PB 8 mg/kg+L-DOPA 300 mg/kg	—	—	15	18
PB 8 mg/kg+L-5HTP 300 mg/kg	—	—	16	14
PB 20 mg/kg+L-DOPA 300 mg/kg	1	20	—	—
PB 20 mg/kg+L-5HTP 300 mg/kg	2	20	—	—

Maximal electroshock (MES) seizure; number of tonic extensor positive mice, 20 mice in one group. Pentylenetetrazole (PTZ) seizure; number of clonic convulsion positive mice, 20 mice in one group, normal, PTZ 100 mg/kg *i.p.*, reserpinized, PTZ 70 mg/kg *i.p.*.

L-DOPA or L-5HTP in 0.3% CMC suspension was orally (*p.o.*) administered in a volume of 0.2 ml/mouse. DPA was *i.p.* given after 40 min of L-DOPA or L-5HTP administration, and after 20 min of DPA dosage, the anticonvulsant activity was examined. In the case of the combined administration of PB with L-DOPA or L-5HTP, PB was *i.p.* given immediately after L-DOPA or L-5HTP dosage, and after 60 min, the anticonvulsant activity was examined.

5HT and 5HIAA in the Brain, and Tryptophan and DPA in the Serum(Table II)

The effects of DPA and PB on those objects were examined. The dose of DPA, 250 mg/kg, was the same as that of median effective dose to MES seizure in the normal,[10] and the minimal effective dose to MES seizure in the reserpinized(Table I). The dose of PB, 40 mg/kg, was 2.5 times higher than that of median effective dose to MES seizure in the normal.[10]

DPA elevated the cerebral 5HIAA. In the case of combined administration of DPA and tranylcypromine(monoamine oxidase inhibitor), the concentration of 5HT was higher than in single administration of each drug, and that of 5HIAA was higher than in single administration of tranylcypromine. The total tryptophan level in the serum was lowered to 30% of the control by the administration of DPA. However, the concentration of tryptophan in free form was not changed. On the other hand, more than 50% of DPA in the serum was bound to serum protein.

PB showed no effects on the concentrations of 5HT, 5HIAA and tryptophan.

GABA in the Brain(Table III)

There was no difference in cerebral GABA content between two control groups, the normal and the reserpinized. DPA elevated GABA content to 140% and 120% at 0.5 hr and 2 hr respectively. PB showed no effect.

Table II. 5HT and 5HIAA in Brain, and Tryptophan and DPA in Serum of the Reserpinized Mouse

	5HT μg/g wet brain	5HIAA μg/g wet brain	Tryptophan[a] μg/ml	DPA[a] μg/ml
Normal	0.668±0.015	0.339±0.015	11.82±0.65 2.70	—
Control	0.235±0.017	0.337±0.012	12.90±0.75 2.10	—
DPA 250 mg/kg *i.p.* 0.5 hr	0.244±0.012	0.409±0.012[c]	5.01±0.12[c] 2.70	493.0 232.4
DPA 250 mg/kg *i.p.* 2 hr	0.251±0.012	0.509±0.006[c]	4.31±0.21[c] 2.80	243.3 107.5
PB 40 mg/kg *i.p.* 1 hr	0.211±0.018	0.341±0.012	11.30±0.35 2.40	—
PB 40 mg/kg *i.p.* 2 hr	0.213±0.012	0.311±0.012	11.20±0.45 2.60	—
Tranylcypromine sulfate 27 mg/kg[b]	0.877±0.041	0.098±0.005	12.54±0.52 2.33	—
Tranylcypromine sulfate 27 mg/kg[b] + DPA 250 mg/kg	1.177±0.008[d]	0.157±0.005[d]	4.40±0.12 2.43	359.0 167.1
Tranylcypromine sulfate 27 mg/kg[b] + PB 40 mg/kg	0.937±0.024	0.092±0.012	11.73±0.23 2.71	—

The value represents the mean ±S.E. of five samples. Brain or serum samples of three mice were combined for the assay.
a) Upper; total μg/ml Lower; free form μg/ml. The assays of DPA and the free form of tryptophan were carried out using the combined serum of the group.
b) DPA or PB was *i.p.* administered 1 hr after tranylcypromine administration. The mice were decapitated 3 hr after *i.p.* administration of tranylcypromine.
c) Significantly different from the control, $p < 0.01$.
d) Significantly different from the single administration of tranylcypromimine, $p < 0.01$.

Table III. Cerebral GABA Content of Content of the Reserpinized Mouse

	GABA μmol/g wet brain
Normal ($n=9$)	2.24±0.07
Control ($n=10$)	2.34±0.06
DPA 250 mg/kg *i.p.* 0.5 hr ($n=11$)	3.28±0.09[a]
DPA 250 mg/kg *i.p.* 2 hr ($n=11$)	2.84±0.09[a]
PB 40 mg/kg *i.p.* 1 hr ($n=9$)	2.35±0.06
PB 40 mg/kg *i.p.* 2 hr ($n=8$)	2.27±0.05

The value represents the mean ±S.E. of 8—11 mice.
a) Significantly different from the control, $p < 0.01$.

Discussion

In the previous study, we reported that DPA decreased the concentration of serum tryptophan, and increased the cerebral concentrations of tryptophan, 5HT and 5HIAA in the normal rat.[2] From these results, it was proposed that DPA might elevate the cerebral turnover of 5HT by accelerating the incorporation of tryptophan into the brain. Since the effect of DPA on the metabolism of the cerebral 5HT might have a role in its anticonvulsant activity, the studies on the effects of DPA in the reserpinized mice will be useful to investigate the mechanism of its anticonvulsant activity.

Concerning the cerebral 5HT and 5HIAA, and the serum tryptophan, DPA showed similar effects in the reserpinized mouse as in the normal rat.[2] DPA strikingly increased the cerebral 5HIAA, and decreased the serum tryptophan content in the reserpinized mice. When DPA was given together with tranylcypromine, the concentration of the cerebral 5HT was higher than in the case of single administration of DPA or tranylcypromine. Thus, DPA elevated the turnover of the cerebral 5HT as indicated in the previous paper.[2]

There have been some reports that suggested the close relationship between cerebral GABA and convulsion,[11] and that clarified the actions of anticonvulsant drugs to the cerebral GABA.[12] The treatment with reserpine had not changed the concentration of the cerebral GABA, furthermore DPA elevated the GABA content (40% increase to the control at 0.5 hr) in the reserpinized mouse as in the normal rat.[2] These results might suggest that DPA to some extent showed anticonvulsant activity through the elevation of GABA content in the reserpinized mice.

PB did not change the metabolisms of 5HT and GABA. This finding implicates that the anticonvulsant mechanisms of PB and DPA are not similar to each other.

In MES seizure, the efficacies of DPA and PB were lower in the reserpinized mice than in the normal, and hence the cerebral amine might be necessary to show the activities of DPA and PB.

In PTZ seizure, the seizure-protecting activity of DPA was observed in both normal and reserpinized mice. However, the activity of PB was decreased in the reserpinized mice. L-5HTP itself had the seizure-protecting activity in the reserpinized mice. And also, in the case of combined administration of DPA and L-DOPA, the anticonvulsant activity was lower than in the single administration of DPA in the reserpinized mice. From these results, it was supposed that the appearance of the PTZ seizure was influenced by the content of the cerebral 5HT in the reserpinized mice. And as Kishimoto, *et al.*[13] suggested that the metabolism of 5HT was influenced by the metabolisms of cathecolamines in the normal monkey, the anticonvulsant activity of DPA and its effect on the cerebral 5HT might be affected by L-DOPA in the reserpinized mice.

In conclusion, the presented results suggested that DPA showed the anticonvulsant activity partially through its effect on the cerebral metabolism of 5HT as well as GABA.

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