Effects of Sodium Dipropylacetate on γ -Aminobutyric Acid and Biogenic Amines in Rat Brain

KAZUAKI KUKINO and TAKASHI DEGUCHI

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.¹⁾

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The effects of sodium dipropylacetate (DPA) on the metabolisms of γ -aminobutyric acid (GABA) and biogenic amines in the rat brain have been investigated. The results obtained are as follows, which are suggestive of the mechanism of anticonvulsant activity of DPA.

1) The level of GABA in the brain was elevated after oral administration (400 mg/kg). At the peak time (0.5 hr), about 45% increase was observed and the elevation of GABA content was maintained for 4 hr after administration.

2) In the brain slices, DPA elevated GABA content and suppressed the oxygen consumption at 6 mm.

3) DPA had weak inhibitory effects on GABA metabolizing enzymes in brain homogenate.

4) The concentrations of 5-hydroxyindoleacetic acid and tryptophan in the brain were elevated after oral administration of DPA (400 mg/kg). These effects might be caused by the increase of free tryptophan in the serum followed by accelerated incorporation of tryptophan into the brain and the elevated turnover rate of 5-hydroxytryptamine in the brain.

5) DPA elevated the content of dopamine, but the level of norepinephrine was not affected.

The changes of these biogenic substances were coincident with the appearance of the anticonvulsant activity of DPA.

Keywords—anticonvulsant; sodium dipropylacetate; rat brain; γ -aminobutyric acid; oxygen consumption; biogenic amines

Sodium dipropylacetate (DPA), a branched chain carboxylic acid, was shown to possess anticonvulsant activity both in clinical and experimental seizures.²⁾

Concerning the mechanism of action, Godin, *et al.*³⁾ showed the increase of γ -aminobutyric acid (GABA) content by DPA in the rat brain. According to their report, in brain homogenate of the rat, DPA inhibited the GABA metabolizing enzymes, L-glutamate decarboxylase (GAD, EC 4.1.1.15) and 4-aminobutyrate:2-oxo-glutarate aminotransferase (GABA-T, EC 2.6.1.19). The inhibition was more pronounced on the GABA-T and this observation might suggest the explanation of the increase of GABA level *in vivo*. However, the inhibitory concentration was very high (50% inhibition at about 50 mM) and no inhibition was demonstrated when DPA was added *in vivo*. Recently, Fowler, *et al.*⁴⁾ reported DPA had weak inhibitory effect on the partially purified GABA-T obtained from rabbit brain. Thus, the mechanism of elevation of GABA is still unexplained. The present investigation has been undertaken to clarify the effects of DPA on the metabolisms of amino acids and biogenic amines.

Experimental

Animals—Male, Wistar strain rats weighing 200 ± 10 g were fasted for 16 hr prior to the administration of DPA and kept throughout the experiments. In the case of successive administration, the rats were

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fed freely and fasted for 16 hr prior to the last administration. The solution of DPA in distilled water was orally administered by a catheter at 9:00 a.m. and the control group received the same amount of distilled water. The rat was killed by decapitation and the brain was rapidly removed. For the determination of amino acids, the brain was quickly thrown into dry ice-isopentane, and in the cases of biogenic amines, the brain was washed with ice-cold saline before freezing. It took about 30 sec from decapitation to freezing. In all analyses, the cerebellum was discarded.

Preparation of Brain Cortex Slices—The cerebral cortex slices were prepared using hand made slicer.⁵⁾ From each hemisphere two slices, 80–100 mg (wet weight), were obtained and they were not more than 0.4 mm thick. When the slices from the right hemisphere were used as the control group, those from the left were treated with DPA. The slices were suspended in 3 ml of chilled Krebs-Ringer phosphate medium contained in Warburg manometer flasks of the following composition: 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 2.7 mM CaCl_2 , 8.5 mM phosphate buffer pH 7.4, 10 mM glucose or 5 mM GABA as the substrate. To compensate ionic strength, the control flask contained equal molar of NaCl instead of DPA. Incubation was carried out in the Warburg manometric apparatus in the atmosphere of 100% O_2 at 37° for 60–90 min after initial oxygenation (2 min) and thermal equilibration (10 min) periods. The rate of oxygen consumption was read at 5 min intervals.

Chemical Analysis—GABA was analysed by the method of Sandman.⁶⁾ 5-Hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) were determined fluorometrically by the method of Curzon and Green.⁷⁾ Tryptophan was measured by the method of Denckla and Dewey.⁸⁾ The serum was deproteinized by passing through the Diaflo-01-T membrane filter. Dopamine (DA) and norepinephrine (NE) were extracted with hydrochloric acid in *n*-butanol from the brain and determined fluorometrically by the method of Maickel, *et al.* and Schlumpf, *et al.*⁹⁾ DPA was measured with gas-liquid chromatography by the method of Kukino, *et al.*¹⁰⁾ For the determination of amino acids, the brains were deproteinized with 75% ethanol solution in water and the free amino acids were analyzed with JEOL-6AH amino acid analyzer.

Enzyme Assays—The activity of GAD in the brain homogenate was measured by the method of Godin, *et al.*³⁾ using ^{14}C -(U)-glutamate. $^{14}\text{CO}_2$ produced throughout the reaction was absorbed in 0.3 ml of ethanolamine mixture (ethanolamine:methanol/1:1). The activity of GABA-T was determined by the production of glutamate from α -ketoglutarate according to the method of Baxter and Roberts.¹¹⁾

Results

GABA Contents

Table I shows the GABA contents after single or successive administration of DPA. DPA elevated the GABA content rapidly after the administration. At the peak time (0.5 hr),

TABLE I. Effect of DPA on GABA Content in Brain

Time after administration hr	Control	0	0.25	0.5
Single administration $\mu\text{mol/g}$	1.78 ± 0.05	—	2.14 ± 0.17	$2.58 \pm 0.09^a)$
Successive administration $\mu\text{mol/g}$	1.89 ± 0.06	$1.78 \pm 0.05^a)$	—	$2.59 \pm 0.08^{a,c)}$

Time after administration hr	1	2	4	6	8
Single administration $\mu\text{mole/g}$	$2.45 \pm 0.05^a)$	$2.41 \pm 0.08^a)$	1.91 ± 0.04	1.93 ± 0.08	1.80 ± 0.06
Successive administration $\mu\text{mole/g}$	$2.58 \pm 0.08^{a,c)}$	—	—	—	—

The value represents the mean $\pm$ S.E. of five rats.

DPA was orally administered in 400 mg/kg, and the same dose was successively administered one shot a day for 11 days.

a) Significantly different from the control, $p < 0.01$.

b) Value after 24 hr of 10th administration.

c) Value after 11th administration.

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about 45% increase of GABA content was observed in either administration schedule. The elevated level of GABA was maintained at least for 4 hr after single administration and the level was completely recovered around 8 hr later. Furthermore, in case of successive administrations, the elevation was the same level as the single administration and not lasted for 24 hr.

O₂ Consumption in Slices

Fig. 1 shows O₂ consumption in the brain slices. The time courses were different between two substrates. In glucose, O₂ consumption increased linearly during the incubation, however in GABA, the rate of O₂ consumption gradually decreased. DPA exerted inhibitory effects on the O₂ consumption at 6 mM (1 mg/ml) in both substrates and no inhibitory effects were found at 1.2 mM (0.2 mg/ml). In addition, these inhibitory effects were not observed in the mixtures containing 1 mM pyridoxal phosphate.

Fig. 1. Effect of DPA on O₂ Consumption in the Brain Slices

Each point represents the mean value of three experiments. The substrates were 10 mM glucose (A) and 5 mM GABA (B).

○—○, control; ●—●, +6 mM DPA, △—△, control; ▲—▲, +6 mM DPA.

TABLE II. Contents of the Amino Acids in Brain and Slice

	Brain μmol/g		Slice μmol/g	
	Control	DPA 400 mg/kg	Control	DPA 6 mM
GABA	1.61±0.02	1.96±0.02 ^{a)}	0.92	1.55
Aspartate	2.66±0.03	1.39±0.03 ^{a)}	1.80	2.72
Glutamate	10.86±0.18	11.23±0.22	4.79	5.29
Glutamine	4.07±0.13	4.64±0.10	1.21	1.45
Glycine	0.79±0.01	0.85±0.01	0.38	0.55

The value represents the mean ± S.E. of three brains or two experiments of the slices. The brains were obtained 0.5 hr after oral administration of DPA. The slices were incubated in 10 mM glucose as the substrate for 1 hr and the slices in two flasks were combined for the assay.

^{a)} Significantly different from the control, $p < 0.01$.

Amino Acids

The main five amino acids were analysed (Table II) and the others were too little to determine quantitatively. Both in the brains and the slices, the GABA contents of the group treated with DPA were higher than those of the control. On the other hand, the aspartate content in the brain treated with DPA was lower than that of the control. However both in the brain and the slice, the levels of the other three amino acids in the group treated with DPA were the same as those in the control group.

Activity of GABA Metabolizing Enzymes

The effects of DPA on the GABA metabolizing enzymes at their optimum pH were shown in Table III. There found no changes at low DPA concentrations, but at a relatively high concentration (60 mM) the inhibitory effects on the both enzymes were observed. Pyridoxal

phosphate reduced to some extent the inhibitory effect of DPA on GAD, however in regard to GABA-T, did not show any effects on the enzyme activity.

TABLE III. Effects of DPA on GAD and GABA-T in Brain Homogenate

Enzyme pH of reaction medium	GAD 6.5				GABA-T 8.2			
	0		1		0		1	
	0.5	1	0.5	1	0.5	1	0.5	1
Pyridoxal phosphate mm								
Incubation time hr								
Control	100 (2200)	100 (3200)	100 (4200)	100 (8900)	100 (230)	100 (285)	100 (595)	100 (660)
DPA 1.2 mM	91	100	100	100	100	100	97	95
DPA 6.0 mM	83	100	98	99	98	95	95	92
DPA 60.2 mM	33	35	60	59	90	91	86	89

The value represents relative percentage to the control, and the mean of four experiments (two tubes per experiment). The values in parentheses were d.p.m. of produced $^{14}\text{CO}_2$ for GAD and μg of glutamate for GABA-T. For the assay of GAD, 1 μCi of ^{14}C -(γ)-glutamate was added in each tube.

5-Hydroxytryptophan and 5-Hydroxyindoleacetic Acid

Table IV shows the contents of 5HT and 5HIAA in the brain after single administration of DPA. DPA caused a little rise of 5HT 0.5 hr (about 10% to the control), and marked increase in 5HIAA (about 150% to the control 2 hr) throughout the experiment. The effect of DPA on the level of 5HIAA appeared later than the effect on 5HT.

TABLE IV. Effects of DPA on 5HT and 5HIAA in Brain

Time after administration hr		0.5	1	2	4	6
Control $\mu\text{g/g}$	5HT	0.67 $\pm$ 0.01	0.64 $\pm$ 0.01	0.74 $\pm$ 0.04	0.77 $\pm$ 0.02	0.72 $\pm$ 0.05
	5HIAA	0.46 $\pm$ 0.01	0.57 $\pm$ 0.03	0.47 $\pm$ 0.01	0.60 $\pm$ 0.03	0.60 $\pm$ 0.02
DPA 400 mg/kg oral administration $\mu\text{g/g}$	5HT	0.76 $\pm$ 0.01 ^{a)}	0.70 $\pm$ 0.01	0.75 $\pm$ 0.02	0.78 $\pm$ 0.03	0.77 $\pm$ 0.01
	5HIAA	0.68 $\pm$ 0.04	1.04 $\pm$ 0.06 ^{a)}	1.17 $\pm$ 0.06 ^{a)}	1.30 $\pm$ 0.04 ^{a)}	1.17 $\pm$ 0.06 ^{a)}

Mean $\pm$ S.E. of five rats.

^{a)} Significantly different from the control, $p < 0.01$.

Tryptophan in the Brain and the Serum

The analyses of the levels of tryptophan were also carried out (Table V), DPA significantly increased the level of total tryptophan in the brain. The effect was maintained for

TABLE V. Effects of DPA on Contents of Tryptophan in Brain and Serum

Time after administration hr		0.5	1	2	4	6
Control	Brain $\mu\text{g/g}$	2.2 $\pm$ 0.1	2.3 $\pm$ 0.1	2.3 $\pm$ 0.1	2.5 $\pm$ 0.1	2.8 $\pm$ 0.1
	Serum ^{a)} $\mu\text{g/ml}$	23.6 $\pm$ 0.7	21.3 $\pm$ 0.3	22.1 $\pm$ 0.8	23.5 $\pm$ 0.6	24.2 $\pm$ 0.5
		2.1	2.5	3.6	2.2	4.0
DPA 400mg/kg oral administration	Brain $\mu\text{g/g}$	5.5 $\pm$ 0.2 ^{b)}	5.0 $\pm$ 0.2 ^{b)}	4.6 $\pm$ 0.3 ^{b)}	3.6 $\pm$ 0.2	2.7 $\pm$ 0.1
	Serum ^{a)} $\mu\text{g/ml}$	5.1 $\pm$ 0.4 ^{b)}	5.4 $\pm$ 0.3 ^{b)}	5.7 $\pm$ 0.6 ^{b)}	7.5 $\pm$ 1.0 ^{b)}	15.6 $\pm$ 0.7
		4.7	4.4	4.3	5.9	3.6
	DPA in serum ^{a)}	1155	985	746	434	119
	$\mu\text{g/ml}$	107	112	110	67	33

The value represents the mean $\pm$ S.E. of five rats.

The assays of the free form were carried out using the combined serum of five rats.

^{a)} Upper: total $\mu\text{g/ml}$ lower: free form $\mu\text{g/ml}$.

^{b)} Significantly different from the control, $p < 0.01$.

4 hr after administration, and the level recovered to that of the control 6 hr. While, the level of total tryptophan in the serum was greatly decreased (25% of the control 0.5 hr), greater part of which consisted of the free form. The almost all molecules of DPA in the serum were bound to the serum proteins. In accordance with the decrease of DPA in the serum, the level of tryptophan increased or decreased, in the serum or in the brain respectively.

Tryptophan Bound to the Serum Proteins *in Vitro*

In order to clarify the possibility that DPA competed with tryptophan for the binding sites in the serum proteins, DPA was incubated with rat serum. The results shown in Table VI indicated that DPA (up to 500 $\mu\text{g/ml}$) had the competitive activity for the binding sites, and increased the concentration of free tryptophan. While, the level of free tryptophan scarcely changed when the concentration of DPA was raised from 500 $\mu\text{g/ml}$ to 1000 $\mu\text{g/ml}$, no matter the bound form of DPA was increased proportionally.

TABLE VI. Effect of DPA on Binding of Tryptophan to Serum Protein

DPA $\mu\text{g/ml}$	0	200	500	1000
Total tryptophan $\mu\text{g/ml}$	29.9	30.1	29.0	29.7
Tryptophan in free form $\mu\text{g/ml}$	2.4(8)	6.7(22)	14.3(49)	14.4(48)
Tryptophan in bound ^{a)} form $\mu\text{g/ml}$	27.5	23.4	14.7	15.3
DPA in free form $\mu\text{g/ml}$	0	15	92	137
DPA in bound form ^{a)} $\mu\text{g/ml}$	0(0)	185(93)	408(82)	863(86)

The value represents the mean of two experiments, and the values in parentheses are relative percentages to total amount of tryptophan or DPA. The rat serum was incubated 1 hr and treated with Diaflo-OI-T membrane filter.

a) Total μg minus free form μg .

Dopamine and Norepinephrine

The contents of DA and NE in the brain after single administration of DPA were shown in Table VII. The level of DA increased 0.5 and 1 hr after administration, however, no change was observed in the level of NE.

TABLE VII. Effects of DPA on DA and NE in Brain

Time after administration hr		0.5	1	2	4	6
Control $\mu\text{g/g}$	DA	1.49 ± 0.10	1.53 ± 0.02	1.37 ± 0.04	1.27 ± 0.10	1.49 ± 0.09
	NE	0.55 ± 0.01	0.60 ± 0.01	0.50 ± 0.03	0.39 ± 0.02	0.58 ± 0.02
DPA 400mg/kg oral administration $\mu\text{g/g}$	DA	$2.06 \pm 0.06^a)$	1.82 ± 0.05	1.36 ± 0.02	1.30 ± 0.09	1.35 ± 0.08
	NE	0.59 ± 0.02	0.57 ± 0.01	0.46 ± 0.01	0.38 ± 0.01	0.59 ± 0.02

Mean $\pm$ S.E. of five rats.

a) significantly different from the control, $p < 0.01$.

Discussion

DPA elevated the levels of GABA in the brain and the slices. As for the mechanism of the elevation, Godin, *et al.*³⁾ mentioned that the inhibitory activity of DPA at high concentrations was more pronounced on the GABA degrading enzyme (GABA-T) than on the GABA producing enzyme (GAD) in brain homogenate. It has been also confirmed in the present study (Table III) that DPA had quite weak inhibitory activity on the enzymes even

at high concentration in brain homogenate (60 mM: ten times higher than the concentration in the serum at the peak time *in vivo*). Thus DPA might not be an inhibitor of the enzymes and the mechanism of GABA elevation was not explained yet.

On the other hand, some suggestive results have been obtained in the present investigation. DPA suppressed the oxygen consumption in the brain slices and this suppression was diminished when 1 mM pyridoxal phosphate was added in the incubation mixtures (Fig. 1). DPA suppressed the formation of aspartate *in vivo* and the degradation in the slice (Table II). From these results, it was suggested that DPA might have the effect on TCA-cycle or on the enzymes depended on pyridoxal phosphate such as aspartate aminotransferase (E.C. 2.6.11). Since the metabolism of GABA closely joined to TCA-cycle as GABA-shunt, the level of GABA might be changed indirectly through these effects of DPA. Furthermore, DPA was found to be metabolized as a carboxylic acid through β and ω oxidation in the rat,¹²⁾ there might be another possibility that some of these metabolites had the effects on TCA-cycle including GABA metabolizing enzymes.

DPA elevated the contents of 5HIAA and tryptophan in the brain (Table IV, V). These effects might be caused by the increase of free tryptophan in the serum followed by accelerated incorporation of tryptophan into the brain and the elevated turnover rate of 5HT in the brain. DPA also elevated DA in the brain. Thus the levels of biogenic amines were greatly affected by the administration of DPA.

There have been many reports that suggested the role of GABA as an inhibitory transmitter and the relationship between neuronal excitability and GABA, while the relationship between convulsion and biogenic amines in the brain remains to be elucidated. However, some benzodiazepine derivatives and diphenylhydantoin were reported to elevate 5HT level in the mouse brain.¹³⁾ And DL-dihydroxyphenylalanine or DL-5-hydroxytryptophan had the anticonvulsant activity in the mouse and the rat, which were pretreated with reserpine and lowered the threshold to electrical and audiogenic seizures.¹⁴⁾ In addition, sodium salicylate, which elevated the turnover rate of 5HT in the brain¹⁵⁾ had the weak activity of anti-maximal electronic seizure in the rat.¹⁶⁾ Furthermore, the appearance of the anticonvulsant activity of DPA¹⁷⁾ was coincident with the changes of those biogenic amines. Therefore, there might be some relationship between the anticonvulsant activity of DPA and its effects on the brain amines.

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