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General Pharmacological Actions of N-Acetoacetyl-3-hydroxytyrosine[1]

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The general pharmacological actions of N-acetoacetyl-3-hydroxytyrosine (L-DOPA acetoacetate) were investigated and compared with those of L-3,4-dihydroxypenylalanine (L-DOPA). The following results were obtained.

1) L-DOPA acetoacetate and L-DOPA suppressed slightly the spontaneous motor activity in mice.

2) L-DOPA acetoacetate and L-DOPA showed no effect on the sleeping time induced by hexobarbital and on maximal electroshock-, pentetrazol- and strychnine-convulsions in mice, but maximal electroshock convulsion was inhibited by the combined administration of L-DOPA and a monoamine oxidase inhibitor, pargyline.

3) L-DOPA acetoacetate and L-DOPA suppressed the squirming induced by acetic acid in mice.

4) L-DOPA acetoacetate did not have a hypothermic action in mice although L-DOPA did slightly.

5) In anaesthetized rats, L-DOPA acetoacetate and L-DOPA produced a slight temporary rise in the blood pressure and potentiated the pressor actions of norepinephrine and tyramine. The tyramine-induced tachyphylaxis and the abolishment of the response of tyramine induced with reserpine pretreatment were restored by both compounds.

6) On the isolated smooth muscle preparations such as guinea-pig vas deferens, intestine, aorta, rabbit aorta and rat uterus, the contractions induced by several agonists were almost unaffected by L-DOPA acetoacetate and L-DOPA. On guinea-pig vas deferens, aorta and rabbit aorta preparations, however, the tyramine-induced tachyphylaxis and the abolishment of the response of tyramine induced with reserpine pretreatment were restored by both compounds. These restorations were not observed by the treatment of a peripheral DOPA decarboxylase inhibitor, Ro4-4602. These actions were also found on guinea-pig atria preparations. Furthermore, on rat uterus preparations, both compounds increased the spontaneous motility.

7) On the gastrointestinal propulsion in mice and on the isolated skeletal muscle preparations in frog, L-DOPA acetoacetate and L-DOPA showed no effect.

From the results mentioned above, it could be assumed that L-DOPA acetoacetate possessed only peripheral without central action though L-DOPA possessed both of central and peripheral actions, and that the activities would be due to their metabolites.

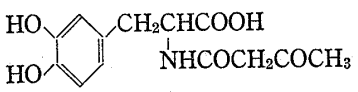
It has been reported that the contents of dopamine (DA) in the basal ganglia and the corpus striatum are markedly decreased in patients with Parkinson's disease induced by damage of the extrapyramidal system.[3] The application of DA, therefore, was conceived as a method for the treatment of Parkinson's disease. Since DA cannot pass the blood-brain-barrier, L-3,4-dihydroxyphenylalanine (L-DOPA) which is a precursor of DA was used in treating the disease.[4]

1) This work was reported at Meeting of Tohoku Branch, Pharmaceutical Society of Japan, Sendai, October 1973.
2) Location: *Aobayama, Sendai, 980, Japan.*
3) H. Ehringer and O. Hornykiewicz, *Klin. Wochenschr.*, **38**, 1236 (1960).
4) G.C. Cotzias, M.H. Van Woert, and L.M. Schiffer, *New Engl. J. Med.*, **276**, 374 (1967); G.C. Cotzias, P.S. Papavasilion, and R. Gellene, *ibid.*, **280**, 337 (1969); A. Barbeau, *Can. Med. Assoc. J.*, **101**, 791 (1969); M.D. Yahr, R.C. Duvoisin, M.M. Hoehn, M.J. Schear, and R.E. Barrett, *Trans. Amer. Neurol. Ass.*, **93**, 56 (1968); D.B. Calne, A.S. Spiers, G.M. Stern, D.R. Lawrence, and P. Armitage, *Lancet*, **2**, 973 (1969); H.L. Klawans and J.S. Garvin, *Dis. Nerv. Syst.*, **30**, 737 (1969); S. Stellar, S. Mandell, J.M. Waltz, and I.S. Cooper, *J. Neurosurg.*, **32**, 275 (1970); C, Maudsley, *Brit. Med. J.*, **1**, 331 (1970).

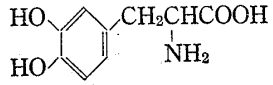
In the present paper, the authors investigated the general pharmacological actions of a derivative of L-DOPA, N-acetoacetyl-3-hydroxytyrosine (L-DOPA acetoacetate), which has an acetoacetyl radical on the N of L-DOPA. The results were compared with those obtained using L-DOPA.

Materials

Test Compounds——



N-acetoacetyl-3-hydroxytyrosine
(L-DOPA acetoacetate)
mp 159—161°



L-3,4-dihydroxyphenylalanine
(L-DOPA)

For preparation of solutions of L-DOPA acetoacetate (for doses of 1—100 mg/kg) and L-DOPA (for doses of 1—10 mg/kg), both compounds were dissolved in 0.9% saline solution. For dose of 100 mg/kg of L-DOPA, a 0.3% CMC suspension was used. These compounds were injected subcutaneously (*s.c.*), intraperitoneally (*i.p.*) and intravenously (*i.v.*) in intact animals, and were applied into each nutritive solution in the experiments of *in vitro*.

Drugs——Other drugs used in experiments were as follows: Hexobarbital-Na, pargyline hydrochloride, pentetrazol, strychnine nitrate, acetic acid, acetylsalicylic acid, salicylic acid, aminopyrine, *dl*-norepinephrine hydrochloride (NE), tyramine hydrochloride, reserpine, acetylcholine chloride (ACh), histamine hydrochloride, barium chloride ($BaCl_2$), nicotine bitartrate, serotonin creatininephosphate (5-HT), *l*-epinephrine hydrochloride (Epi), oxytocin, *d*-tubocurarine chloride (*d*-Tc), succinylcholine chloride (SCh) and N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (Ro4-4602).

Animals——Mouse (ddY strain), rat (Wistar strain), guinea-pig (Hartley strain), rabbit, frog (*Rana nigromaculata*).

Methods

1. Effects on Motor Activity——a) Wheel Cage Method: A wheel cage apparatus was used for the measurement of spontaneous activity, as described by Kameyama, *et al.*[5] Mice (♂, 18—22 g) were placed in the apparatus for 30 min and those mice which showed about 500 revolutions per 30 min were picked out and used in groups of five mice. Test compounds were administered *s.c.* and motor activity measured at 30 min intervals for 150 min.

b) Rotating Rod Method: Groups of six mice (♂, 18—22 g) were placed on a wooden rod rotating around a horizontal axis (3 cm in diameter, 5 rotations per min) and the frequency which the mice fell off from the rod was evaluated. The measurement was carried out for 10 min at 0, 30, 60, 90, and 120 min after the *s.c.* administrations of test compounds.

2. Effects on Hexobarbital Sleeping Time——Groups of five mice (♂, 18—22 g) were given *i.p.* injections of hexobarbital-Na (70 mg/kg) 30 min after the *s.c.* administration of test compounds. Sleeping time was measured as the period between disappearance and restoration of the righting reflex.

3. Anticonvulsant Activity——a) Effects on Maximal Electroshock Convulsion: Groups of five mice (♂, 18—22 g) were used. Maximal electroshock (1.25 mA, 50 V, for 0.8 sec) was applied to the ear-wing of mice 1 hr after *s.c.* administrations of test compounds, and the duration of each tonic flexor (TF), tonic extensor (TE) and clonic convulsion (CL) was measured. Also, mice which were treated with a monoamine oxidase (MAO) inhibitor, pargyline-HCl (100 mg/kg, *i.p.*), 2 hr before the injections of test compounds were used.

b) Effects on Pentetrazol Convulsion: Groups of five mice (♂, 18—22 g) were used. At 15 min after *i.p.* injections of the test compounds, pentetrazol (90 mg/kg) was administered *s.c.*, and the session time of the tonic extension was measured.

c) Effects on Strychnine Convulsion: Groups of five mice (♂, 18—22 g) were used. At 15 min after *i.p.* injections of the test compounds, strychnine (1 mg/kg) was administered *s.c.* and the lethal time measured.

4. Analgesic Activity——a) Acetic Acid Method: Experiments were carried out according to the method of Koster.[6] Groups of five mice (♂, 18—22 g) were used. Thirty min after *s.c.* injections of the test compounds, 0.6% acetic acid in a volume of 0.1 ml/10 g was given *i.p.* The mice were placed in a five-compartment observation cage and the number of squirming for each animal was counted for 20 min after the injection of acetic acid. An inhibitory rate was calculated by comparing the squirming number with controls.

5) T. Kameyama, K. Sasaki, and K. Kisara, *Ann. Rept. Tohoku Coll. Pharm.*, 11, 105 (1963).
6) R. Koster, *Federation Proc.*, 22, 249 (1969).

b) Pressure Method: The experiment was carried out according to the method described by Takagi, *et al.*[7] Before experiments, the normal pain threshold of a number of mice was measured twice and those which did not show a normal pain threshold ranging from 40 to 80 mmHg were eliminated. Groups of five selected mice (♂, 18—22 g) were used. Test compounds were administered *s.c.* and the change in pain threshold measured at 15 min interval for 120 min.

5. Hypothermic Action——Groups of five mice (♂, 18—22 g) were used. Before experiments, normal rectal temperatures were measured and mice showing rectal temperature of 37.5—38.5° were used. Test compounds were administered *s.c.* and the rectal temperature measured at 15 min interval for 6 hr using a thermister (NIHON KOHDEN MGA III-219).

6. Effects on Rat Blood Pressure——Rats (200—400 g) were anaesthetized with 1.4 g/kg of urethane *s.c.* The blood pressure was recorded on a kymograph through a mercury manometer from a carotid artery and drugs were injected into a juglar vein.

7. Effects on Isolated Smooth Muscle Preparation——a) Guinea-pig Hypogastric Nerve-Vas Deferens Preparation: After the vas deferens was dissected together with the hypogastric nerve from guinea-pigs (♂, 300—400 g) according to the method described by Huković,[8] the preparation was suspended in a 10 ml bath containing Tyrode solution at 32° and aerated with 95% O_2+5% CO_2. Contractions of the vas deferens induced by electrical stimulation, NE, ACh and tyramine were recorded on a rectigraph (SANEI 8S) through a transducer or on a kymograph with an isotonic writing lever. Electrical stimulation was applied to the hypogastric nerve at 3 min intervals at a frequency of 50 cps with 1 msec duration and at supramaximal voltage for 3 sec using an electronic stimulator (NIHON KOHDEN MSE-3).

b) Guinea-pig Intestine Preparation: Guinea-pig (300—400 g) intestines were dissected out and suspended in a 10 ml bath containing Tyrode solution at 27° and aerated with 95% O_2+5% CO_2. Contractions were recorded on a kymograph with an isotonic writing lever.

c) Rabbit and Guinea-pig Aorta Preparation: The aortae dissected from rabbits (1—2 kg) and guinea-pigs (300—400 g) were suspended in a 10 ml bath containing Krebs-Henseleit solution (for rabbit aorta) or Tyrode solution (for guinea-pig aorta) at 38° and aerated with 95% O_2+5% CO_2. The contraction was recorded on a rectigraph (SANEI 8S) through a transducer or on a kymograph with an isotonic writing lever.

d) Rat Uterus Preparation: The uterus dissected from the rat (♀, 150—200 g, 14 days after ovariectomy) was suspended in a 10 ml bath containing Locke-Ringer solution at 37° and aerated with 95% O_2+5% CO_2. Spontaneous motility and the contraction of the uterus were recorded on a kymograph with an isotonic writing lever.

8. Effects on Isolated Guinea-pig Atria Preparation——The atria dissected from freshly killed guinea-pigs (400—600 g) were suspended in a 50 ml bath containing Krebs-Henseleit solution at 37° and aerated with 95% O_2+5% CO_2. The contraction and heart rate were recorded on a recticorder (NIHON KOHDEN RJQ-3004) through a transducer.

9. Gastrointestinal Propulsion in Mice——Mice (♂, 18—22 g) fasted 24 hr were used. Ten min after *s.c.* injections of test compounds, 20% $BaSO_4$ solution (0.2 ml/mouse) was administered orally. After 30 min the small intestine was isolated and the length of $BaSO_4$ propulsion for that of the intestine from the pyloric region to the cecum was measured.

10. Effects on Isolated Skeletal Muscle Preparation——a) Frog Sciatic-Sartorius Preparation: The sciatic-sartorius preparation dissected from the frog (25—35 g) was suspended in a 10 ml bath containing Ringer solution at 20° and aerated with air. The twitch of the sartorius muscle induced by the electrical stimulation of the sciatic nerve was recorded on a kymograph with an isotonic writing lever. The electrical stimulation was applied at a frequency of 0.1 cps with 0.4 msec duration and at submaximal voltage.

b) Frog Rectus Adominis Muscle Preparation: The rectus abdominis muscle dissected from the frog (25—35 g) was suspended in a 10 ml bath containing Ringer solution at 20° and aerated with air. The contraction was recorded on a kymograph with an isotonic writing lever.

Results

1. Effects on Motor Activity

a) Wheel Cage Method——The results are shown in Fig. 1. The motor activity was reduced by L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) and L-DOPA (1—100 mg/kg, *s.c.*). These was a slight reduction about 2hr after administration of L-DOPA acetoacetate and an initial reduction after the administration of L-DOPA.

7) K. Takagi, T. Kameyama, and K. Yano, *Yakugaku Zasshi*, **78**, 553 (1968).
8) S. Huković, *Brit. J. Pharmacol.*, **16**, 188 (1961).

b) **Rotating Rod Method**——Falling of mice from the rotating rod was not observed after treatment with L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) or L-DOPA (1—100 mg/kg, *s.c.*).

2. Effects on Hexobarbital Sleeping Time

L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) and L-DOPA (1—100 mg/kg, *s.c.*) did not have hypnotic action. The sleeping time induced by hexobarbital, also, was not potentiated by these compounds.

3. Anticonvulsant Activity

a) **Effects on Maximal Electroshock Convulsion**——The results were shown in Table I. L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) and L-DOPA (1—100 mg/kg, *s.c.*) did not protect mice against maximal electroshock seizures. However after pretreatment with a MAO inhibitor, pargyline (100 mg/kg, *i.p.*), L-DOPA (10—100 mg/kg, *s.c.*) produced a consistent anticonvulsant effect which was particularly remarkable at a dose of 100 mg/kg. After pargyline, on the other hand, L-DOPA acetoacetate did not have any anticonvulsant activity and showed a tendency to extend the time of TE phase.

b) **Effects on Pentetrazol Convulsion**——Results are shown in Table II. The session time of TE was unaffected by L-DOPA acetoacetate (1—100 mg/kg, *i.p.*) and L-DOPA (1—100 mg/kg, *i.p.*).

c) **Effects on Strychnine Convulsion**——Results are shown in Table III. The lethal time was slightly but significantly extended by L-DOPA acetoacetate (1 mg/kg, *i.p.*) and L-DOPA (1 and 100 mg/kg, *i.p.*), but anti-strychnine activity was not observed by either compound.

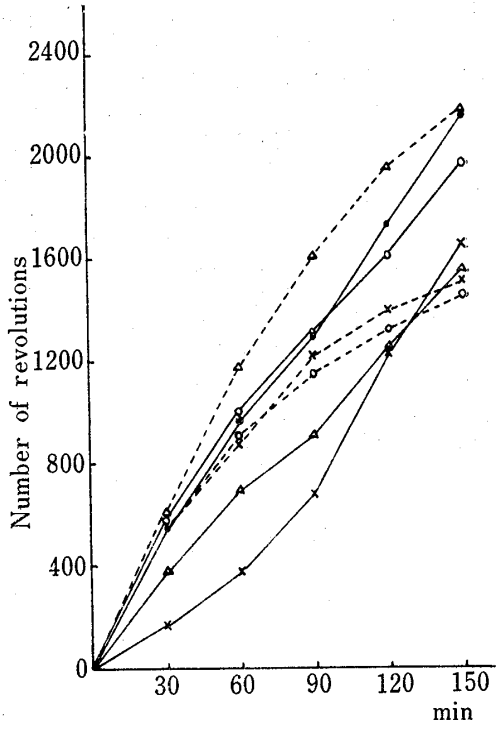


Fig. 1. Effects of L-DOPA Acetoacetate and L-DOPA on Revolution Activity in the Wheel Cage on Mice

Each point is the mean of three groups.
—●—: control
—○—: L-DOPA 1 mg/kg
—△—: L-DOPA 10 mg/kg
—×—: L-DOPA 100 mg/kg
---○---: L-DOPA acetoacetate 1 mg/kg
---△---: L-DOPA acetoacetate 10 mg/kg
---×---: L-DOPA acetoacetate 100 mg/kg

4. Analgesic Activity

a) **Acetic Acid Method**——As shown in Table IV, the squirming number induced by acetic acid was suppressed by L-DOPA acetoacetate (10—100 mg/kg, *s.c.*) and L-DOPA (1—100 mg/kg, *s.c.*). An inhibition of about 50% was observed with 100 mg/kg of both compounds with almost equipotent action when compared to acetylsalicylic acid.

b) **Pressure Method**——The pain threshold was affected only slightly by L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) and L-DOPA (1—100 mg/kg, *s.c.*) (Fig. 2).

5. Hypothermic Action

As shown in Fig. 3, L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) caused no change in normal body temperature, whereas low doses (1 and 10 mg/kg, *s.c.*) of L-DOPA caused a slight and lasting hypothermic action. High doses of L-DOPA (100 mg/kg, *s.c.*) caused a slight and temporary hypothermic action resembling that of aminopyrine.

6. Effects on Rat Blood Pressure

In anaesthetized rats, L-DOPA acetoacetate (10 mg/kg, *i.v.*) and L-DOPA (4 mg/kg, *i.v.*) produced a slight rise in blood pressure. The pressor actions of NE (5 μg/kg, *i.v.*) and tyramine (1 mg/kg, *i.v.*) were potentiated by both compounds (Fig. 4). The tachyphylaxis induced

Table I. Effects of L-DOPA Acetoacetate and L-DOPA on Maximal Electroshock Convulsion in Mice

Drugs	Dose (mg/kg, *s.c.*)	Duration time (sec)		
		TF	TE	CL
a)				
control		2.0	13.0	7.4
L-DOPA acetoacetate	1	2.3	14.3	7.5
	10	2.2	12.0	5.5
	100	2.0	11.9	6.3
L-DOPA	1	2.3	12.3	5.0
	10	2.3	13.3	5.9
	100	2.2	14.2	8.8
b)				
control		1.9	14.9	11.8
L-DOPA acetoacetate	1	2.0	14.3	11.5
	10	1.6	18.1	8.0
	100	1.9	18.9	9.5
L-DOPA	1	2.0	17.0	12.3
	10	3.3	13.3	8.0
	100	—	—	—

Electronic stimulation was applied at 1.25 mA, 0.8 sec and 50 V.
TF: tonic flexor TE: tonic extensor CL: clonic convulsion
Each time is the mean of two groups.
a) L-DOPA acetoacetate and L-DOPA alone
b) 2 hr after the administration of pargyline HCl (100 mg/kg *i.p.*)

Table II. Effects of L-DOPA Acetoacetate and L-DOPA on Pentetrazol Convulsion in Mice

Drugs	Dose (mg/kg, *i.p.*)	Session time of TE	Survival No. used animals
control		6 min 40 sec	3/10
L-DOPA acetoacetate	1	11 13	2/10
	10	10 22	3/10
	100	8 18	3/10
L-DOPA	1	9 29	3/10
	10	9 13	3/10
	100	4 38	3/10

Pentetrazol (90 mg/kg) was administered subcutaneously.

Table III. Effects of L-DOPA Acetoacetate and L-DOPA on Strychnine Convulsion

Drugs	Dose (mg/kg, *i.p.*)	Death time	Survival No. used animals
control		4 min 46 sec	0/10
L-DOPA acetoacetate	1	8 01[a)]	0/10
	10	4 46	2/10
	100	6 41	1/10
L-DOPA	1	6 22[a)]	0/10
	10	5 28	0/10
	100	7 00[a)]	0/10

Strychnine (1 mg/kg) was administered subcutaneously.
a) $p < 0.05$

by the iterative administrations of tyramine (1 mg/kg, *i.v.*) after decreasing to as low as 50% of control was restored with L-DOPA acetoacetate and L-DOPA (Fig. 5). In rats pretreated with reserpine (2 mg/kg/day, *i.p.* for 5 days), the slight pressor actions of both compounds were abolished and the tachyphylaxis by tyramine was restored with both compounds (Fig. 6).

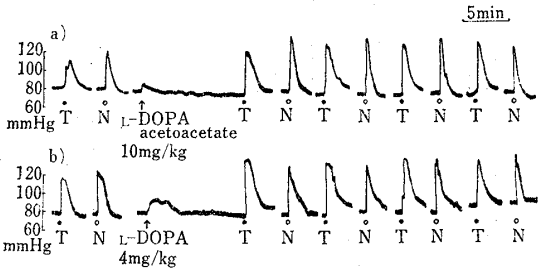


Fig. 6. Effects of L-DOPA Acetoacetate and L-DOPA on Blood Pressure in Reserpinized Rat

●: tyramine 1 mg/kg *i.v.* Reserpine (2 mg/kg/day) was administered intraperitoneally for 5 days. a) L-DOPA acetoacetate (10 mg/kg) and b) L-DOPA (4 mg/kg) were applied at arrow, respectively.

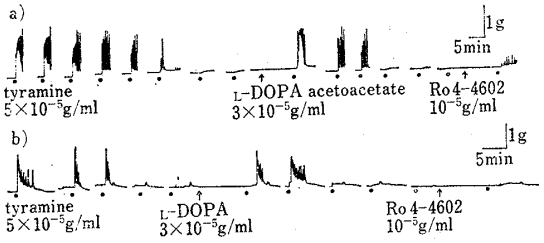


Fig. 7. Effects of L-DOPA Acetoacetate and L-DOPA on Isolated Guinea-pig Vas Deferens Preparation

●: tyramine 5×10^{-5} g/ml ○: Ro4-4602 10^{-5} g/ml a) L-DOPA acetoacetate (3×10^{-5} g/ml) and b) L-DOPA (3×10^{-5} g/ml) were applied at arrow, respectively.

7. Effects on Isolated Smooth Muscle Preparations

a) Guinea-pig Hypogastric Nerve-Vas Deferens Preparation——The contractions of the vas deferens induced by electrical stimulation of the preganglionic fiber of the hypogastric nerve, by NE (10^{-6} g/ml) and by ACh (10^{-6} g/ml) were little affected with L-DOPA acetoacetate (3×10^{-5} g/ml) and L-DOPA (3×10^{-5} g/ml). In vas deferens preparations, the contractions caused by tyramine (5×10^{-5} g/ml) were abolished by the iterative administration of tyramine and by reserpine pretreatment (1 mg/kg/day, *i.p.* for 2 days). After the abolishment of tyramine contraction, these contractions recovered 10 min after the administration of L-DOPA acetoacetate or L-DOPA. When a peripheral DOPA decarboxylase inhibitor, Ro4-4602

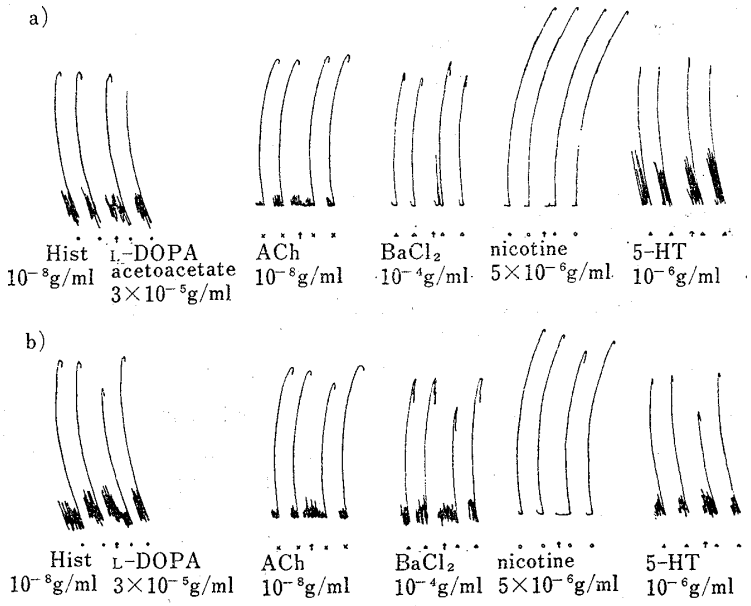


Fig. 8. Effects of L-DOPA Acetoacetate and L-DOPA on Isolated Guinea-pig Intestine Preparation

●: histamine 10^{-8} g/ml, ×: ACh 10^{-8} g/ml, △: $BaCl_2$ 10^{-4} g/ml, ○: nicotine 5×10^{-6} g/ml) ▲: 5-HT 10^{-6} g/ml a) L-DOPA acetoacetate (3×10^{-5} g/ml) and b) L-DOPA (3×10^{-5} g/ml) were applied at arrow, respectively.

TABLE IV. Effects of L-DOPA Acetoacetate, L-DOPA and Other Drugs against Squirming by Acetic Acid in Mice

Drugs	Dose (mg/kg, *s.c.*)	Inhibition (%) ±S.E.[a]
L-DOPA acetoacetate	1	6.9± 1.1
	10	28.7± 5.1[b]
	100	47.3± 8.8[b]
L-DOPA	1	45.5± 6.2[b]
	10	46.3±10.5[b]
	100	50.8± 8.8[b]
Salicylic acid	100	70.0± 8.2[b]
Acetylsalicylic acid	100	45.0± 7.7[b]

a) Inhibitory % was calculated from value compared with control (saline injection group).
b) $p < 0.05$

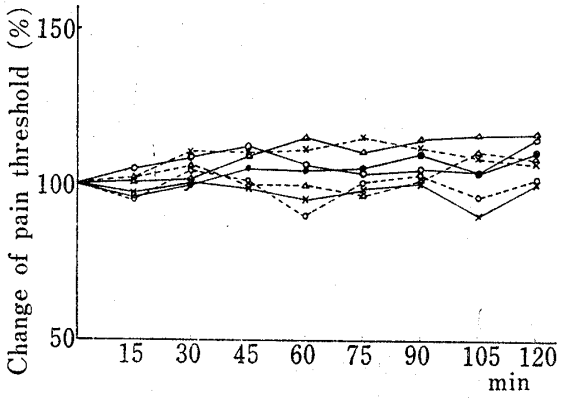


Fig. 2. Effects of L-DOPA Acetoacetate and L-DOPA on Pressure Method in Mice

—●—: control
—○—: L-DOPA acetoacetate 1 mg/kg
—△—: L-DOPA acetoacetate 10 mg/kg
—×—: L-DOPA acetoacetate 100 mg/kg
—○—: L-DOPA 1 mg/kg
---△---: L-DOPA 10 mg/kg
---×---: L-DOPA 100 mg/kg

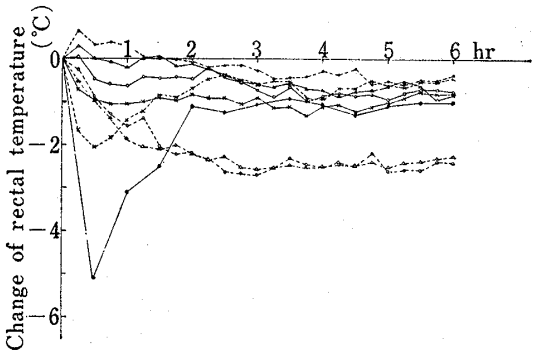


Fig. 3. Effects of L-DOPA Acetoacetate, L-DOPA and Aminopyrine on Normal Rectal Temperature in Mice

---●---: control
—○—: L-DOPA acetoacetate 1 mg/kg
—△—: L-DOPA acetoacetate 10 mg/kg
—×—: L-DOPA acetoacetate 100 mg/kg
---○---: L-DOPA 1 mg/kg
---△---: L-DOPA 10 mg/kg
---×---: L-DOPA 100 mg/kg
—●—: Aminopyrine 100 mg/kg

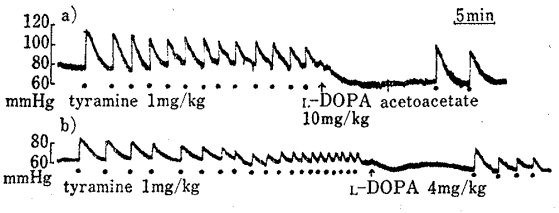


Fig. 4. Effects of L-DOPA Acetoacetate and L-DOPA on Blood Pressure in Anaesthetized Rat

N: norepinephrine 5 μg/kg *i.v.* T: tyramine 1 mg/kg *i.v.*
a) L-DOPA acetoacetate (10 mg/kg) and b) L-DOPA (4 mg/kg) were applied at arrow, respectively.

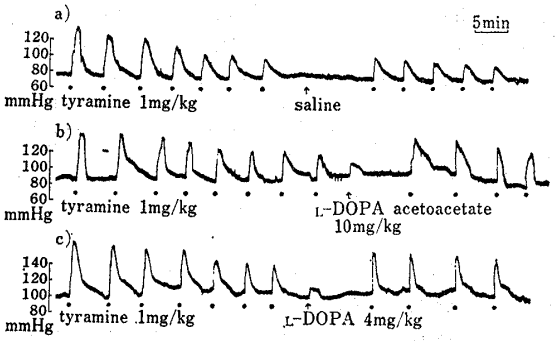


Fig. 5. Effects of L-DOPA Acetoacetate and L-DOPA on Blood Pressure in Anaesthetized Rat

●: tyramine 1 mg/kg *i.v.* a) Saline, b) L-DOPA acetoacetate (10 mg/kg) and c) L-DOPA (4 mg/kg) were applied at arrow, respectively.

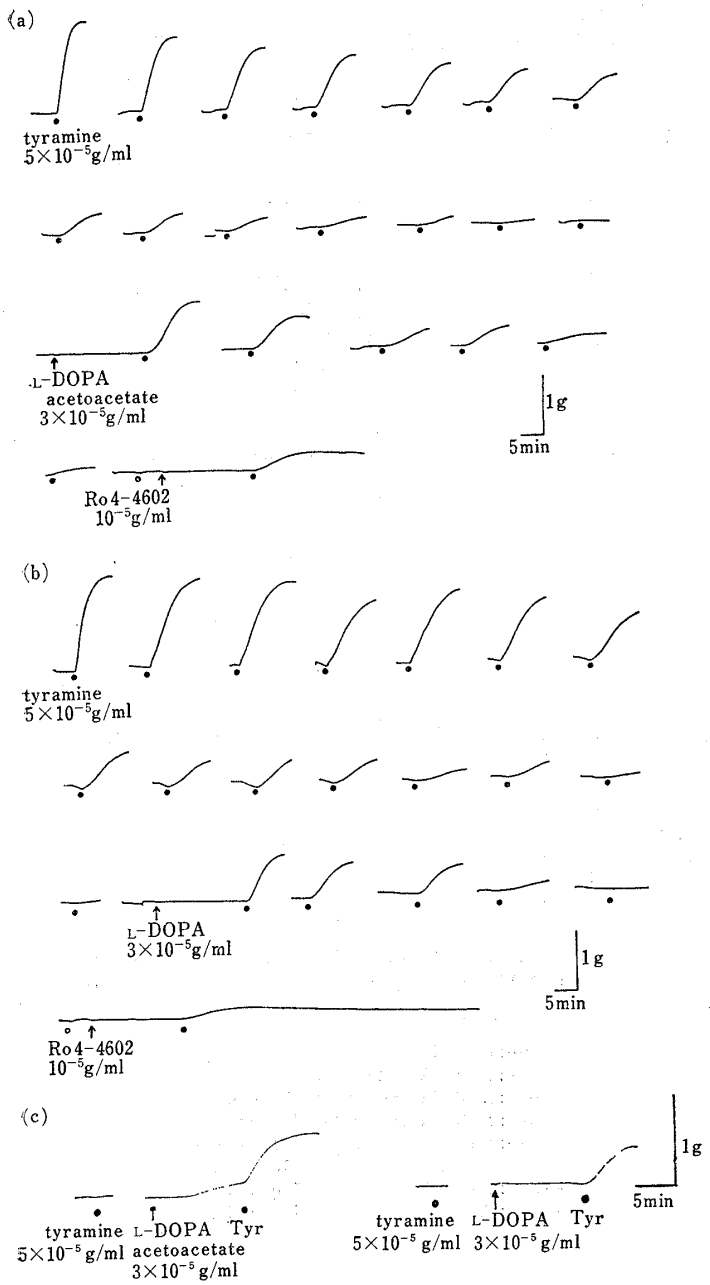


Fig. 9. Effects of L-DOPA Acetoacetate and L-DOPA on Isolated Rabbit and Guinea-pig Aorta Preparations

a) and b) effects of L-DOPA acetoacetate (3×10^{-5} g/ml) and L-DOPA (3×10^{-5} g/ml) on the tachyphylaxis induced by tyramine (5×10^{-5} g/ml) (in rabbit) c) effects of L-DOPA acetoacetate (3×10^{-5} g/ml) and L-DOPA (3×10^{-5} g/ml) on the abolishment of tyramine (5×10^{-5} g/ml) contraction after reserpine pretreatment (1 mg/kg/day, *i.p.* for 2 days) (in guinea-pig).

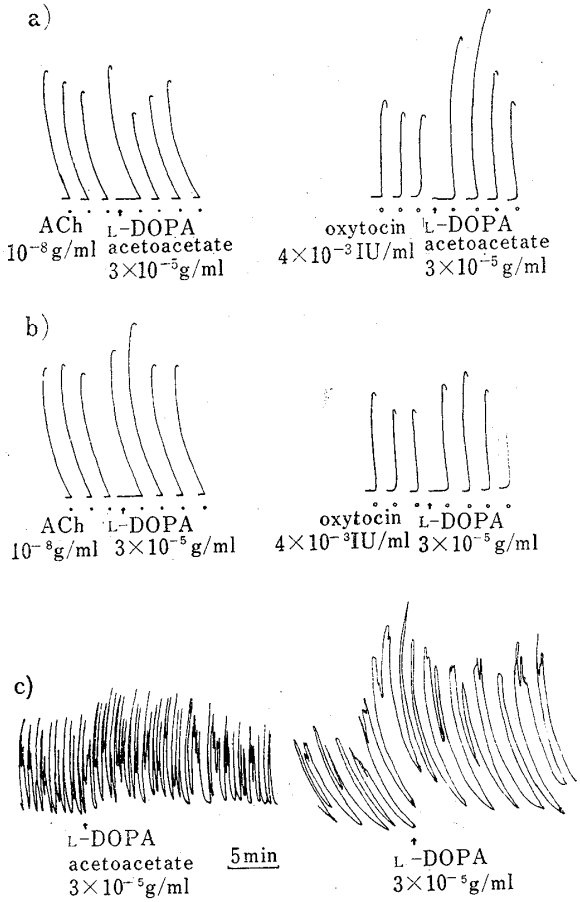


Fig. 10. Effects of L-DOPA Acetoacetate and L-DOPA on Isolated Rat Uterus Preparations

●: ACh 10^{-8} g/ml :oxytocin 4×10^{-3} IU/ml a) L-DOPA acetoacetate (3×10^{-5} g/ml) and b) L-DOPA (3×10^{-5} g/ml) were applied at arrow, respectively c) effects on the spontaneous motility.

(10^{-5} g/ml), was administered before the treatment with either compound, contractions were not observed (Fig. 7).

b) Guinea-pig Intestine Preparation——L-DOPA acetoacetate and L-DOPA at a concentration of 3×10^{-5} g/ml did not possess contractile action in the intestine. L-DOPA suppressed about 20% the contractile responses induced by histamine (10^{-8} g/ml), ACh (10^{-8} g/ml), $BaCl_2$ (10^{-4} g/ml), nicotine (5×10^{-6} g/ml) and 5-HT (10^{-6} g/ml), whereas L-DOPA acetoacetate had no affect on the action of any of these drugs except for about a 15% potentiation of the $BaCl_2$ response (Fig. 8).

c) **Rabbit and Guinea-pig Aorta Preparations**——L-DOPA acetoacetate and L-DOPA at a concentration of 3×10^{-5} g/ml did not cause contraction of the aorta and did not affect the contractile response induced by epinephrine (10^{-5} g/ml) and histamine (5×10^{-5} g/ml). As shown in Fig. 9, the tachyphylaxis induced by the iterative administration of tyramine (5×10^{-5} g/ml) and block of the tyramine (5×10^{-5} g/ml) contraction by reserpine pretreatment (1 mg/kg/day, *i.p.* for 2 days) were restored 10 min after the administration of either compound. These L-DOPA acetoacetate and L-DOPA effects were eliminated by pretreatment with Ro4-4602 (10^{-5} g/ml).

d) **Rat Uterus Preparation**——L-DOPA acetoacetate (3×10^{-5} g/ml) and L-DOPA (3×10^{-5} g/ml) increased the spontaneous motility in diestrus rat uterus, and potentiated the contractions induced by ACh (10^{-8} g/ml) and oxytocin (4×10^{-3} IU/ml) (Fig. 10).

8. Effects on Isolated Guinea-pig Atria Preparation

L-DOPA acetoacetate (3×10^{-5} g/ml) and L-DOPA (3×10^{-5} g/ml) exerted no significant effect on the cardiac contractile force and did not affect the heart rate of isolated atria preparations but did potentiate the NE (10^{-6} g/ml) induced increase in the contractile force of the atria (Fig. 11); restored the tachyphylaxis induced by the iterative administrations of tyramine (10^{-5} g/ml); and abolished of the increase in the contractile force induced by tyramine (10^{-5} g/ml) after reserpine pretreatment (1 mg/kg/day, *i.p.* for 2 days) (Fig. 12). When preparations were treated with Ro4-4602 (10^{-5} g/ml) prior to the administration of either compound, these phenomena were not observed.

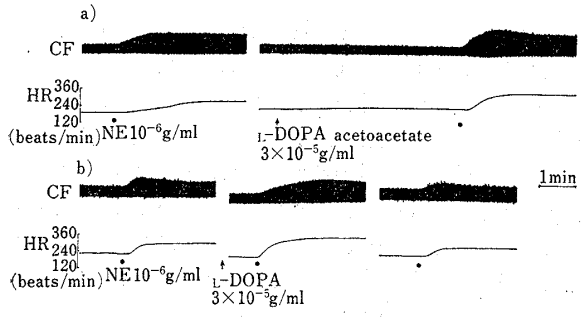


Fig. 11. Effects of L-DOPA Acetoacetate and L-DOPA on Isolated Guinea-pig Atria Preparation

●: norepinephrine (NE, 10^{-6} g/ml) CF: cardiac force HR: heart rate a) effect of L-DOPA acetoacetate (3×10^{-5} g/ml) b) effect of L-DOPA (3×10^{-5} g/ml).

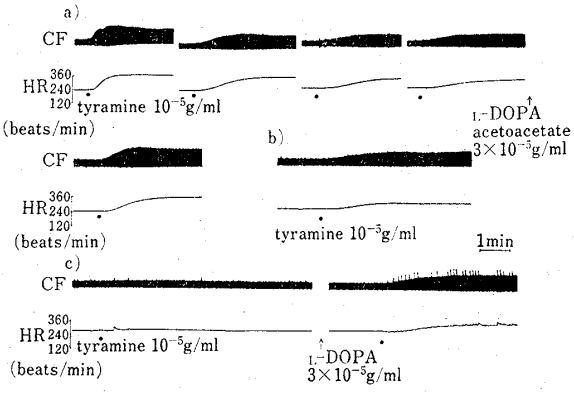


Fig. 12. Effects of L-DOPA Acetoacetate and L-DOPA on Isolated Guinea-pig Atria Preparation

●: tyramine 10^{-5} g/ml a) recovery action of the tyramine-induced tachyphylaxis by L-DOPA acetoacetate (3×10^{-5} g/ml) b) no recovery action of the tyramine-induced tachyphylaxis after using L-DOPA acetoacetate (3×10^{-5} g/ml) and Ro4-4602 (10^{-5} g/ml) together c) effect of L-DOPA (3×10^{-5} g/ml) on the abolishment of tyramine response after reserpine pretreatment (1 mg/kg/day, *i.p.* for 2 days).

TABLE V. Effects of L-DOPA Acetoacetate and L-DOPA on Gastrointestinal Propulsion in Mice

Drugs	Dose (mg/kg, *s.c.*)	Movement in intestine (%) ±S.E.
control		73.7±3.1
L-DOPA acetoacetate	1	71.6±2.5
	10	73.2±4.3
	100	79.5±2.6
L-DOPA	1	73.8±2.1
	10	81.3±2.6
	100	77.1±2.3

9. Gastrointestinal Propulsion in Mice

As shown in Table V, no significant changes in gastrointestinal propulsion were observed with L-DOPA acetoacetate (1—100 mg/kg, *s.c.*) or L-DOPA (1—100 mg/kg, *s.c.*).

10. Effects on Isolated Skeletal Muscle Preparations

a) Frog Sciatic-Sartorius Preparation——The twitches of the sartorius muscle induced by the electrical stimulation of the sciatic nerve were not affected and the muscle relaxing actions induced by *d*-Tc (10^{-6} g/ml) and SCh (5×10^{-6} g/ml) were not antagonized by L-DOPA acetoacetate (3×10^{-5} g/ml) or L-DOPA (3×10^{-5} g/ml).

b) Frog Rectus Abdominis Muscle Preparation——Contractions of the rectus abdominis muscle induced by ACh (10^{-6} g/ml) were not affected by L-DOPA acetoacetate (3×10^{-5} g/ml) or L-DOPA (3×10^{-5} g/ml).

Discussion

Even though it is well known that L-DOPA can be converted metabolically to DA and/or NE, there is some doubts as to whether the pharmacological actions of L-DOPA are due to the action of L-DOPA itself, or due to its metabolites, DA or NE. As mentioned in the introduction L-DOPA is effective for the treatment of Parkinson's disease through the action of synthetized DA. Although it is generally believed that L-DOPA does not have marked pharmacological actions in animals,[9] Kasahara, *et al.*[10] and Hashimoto, *et al.*[11] found that L-DOPA did have some pharmacological activity in the central and peripheral nervous systems. Recently, it has been reported by many investigators that L-DOPA possesses a marked hypotensive action which is likely due to a central mechanism.[12]

In the present paper, the general pharmacological actions of L-DOPA acetoacetate, one of L-DOPA derivatives, were investigated comparing with those of L-DOPA.

The wheel cage method was used to measure motor activity. L-DOPA acetoacetate suppressed slightly motor activity 150 min after its administration, whereas L-DOPA showed a tendency to decrease the activity in the beginning of the administration. On the hexobarbital sleeping time and the various convulsive activities, L-DOPA acetoacetate and L-DOPA showed no effect itself. On the other hand, anti-maximal electroshock convulsion was observed using L-DOPA along with the MAO inhibitor, pargyline HCl. Mckenzie and Soroko[13] observed an anti-convulsive action induced by the use of L-DOPA along with pargyline and they suggested from the species difference that an anticonvulsant activity was related to the central dopaminergic mechanism in rats, but that was not in mice. In contrast to L-DOPA, the use of L-DOPA acetoacetate along with pargyline did not show an anticonvulsant effect in mice. These results may be due to the structural difference between L-DOPA acetoacetate and L-DOPA. The analgesic responses of L-DOPA acetoacetate and L-DOPA recognized by acetic acid stretching method could not give expression to a real analgesic action since the squirming number was decreased also by the drugs possessing sedative action. It may be evident also from the fact that both compounds give no effect on the pain threshold by using

9) H. Blaschko and T.L. Chrusciel, *J. Physiol.* (London), **151**, 272 (1960); O. Hornykiewicz, *Pharmacol. Rev.*, **18**, 925 (1966).
10) A. Kasahara, H. Kojima, T. Hashizume, T. Yamasaki, K. Tawara, and T. Chiba, *Clin. Rep.*, **4**, 131 (1970).
11) T. Hashimoto, K. Kubo, K. Sudo, and M. Tanaka, *Clin. Rep.*, **4**, 148 (1970).
12) M. Henning and A. Rubenson, *J. Pharm. Pharmacol.*, **22**, 242 (1970); D.H. Minisker, A. Scriabine, A.L. Stokes, C.A. Stone, and M.L. Torchiana, *Experientia*, **27**, 529 (1971); R.D. Robson, *Circ. Res.*, **28**, 662 (1971); A.M. Watanabe, L.C. Parks, and I.J. Kopin, *J. Clin. Invest.*, **50**, 1322 (1971); A.M. Watanabe and P.V. Cardon, *Circulation*, **44** (Suppl. 2), 93 (1971); M.W. Osborne, J.J. Wenger, and W. Willems, *J. Pharmacol. Exptl. Therp.*, **178**, 517 (1971); H.R. Kaplan, J.W. Barker, and S.A. La Sala, *European J. Pharmacol.*, **17**, 273 (1972).
13) G.M. Mckenzie and F.E. Soroko, *J. Pharm. Pharmacol.*, **24**, 696 (1972).

pressure method. A hypothermic action was slightly observed by L-DOPA in mice. These results agree with the experimental results in rabbit (above 20 mg/kg, *i.v.*) performed by Takashima.[14] There is a report, however, that a large dose of L-DOPA (above 300 mg/kg) produces rather a hyperthermic action.[10]

From the results mentioned above, it is considered that L-DOPA acts converting to DA or NE in brain through the blood-brain-barrier, whereas that L-DOPA acetoacetate does not possess the central action since it cannot pass the blood-brain-barrier or since it cannot convert to DA acetoacetate even if it can pass the blood-brain-barrier.

In anaesthetized rats, L-DOPA acetoacetate and L-DOPA produced only a slight pressor action, and the action was abolished by pretreatment of reserpine and altered to a slight fall in blood pressure. It has been generally reported that L-DOPA produced a temporary increase followed by a slow decrease in blood pressure which was remarkable with the pretreatments of MAO inhibitors, and that the hypotensive action was mediated centrally.[12] In the present experiments, since only the observation with the short time of the administration of L-DOPA acetoacetate or L-DOPA was carried out, the hypotensive action in normal rats was not recognized.

In blood pressure, aortic strip, vas deferens and atria preparations, after the tyramine-induced tachyphylaxis and the abolishment of the response of tyramine after reserpine pretreatment, these contractions were restored by L-DOPA. These phenomena show that the catecholamine (CA) formed from L-DOPA could be released by tyramine. It must be considered that also the restoration of the tyramine-induced tachyphylaxis induced by L-DOPA acetoacetate is contribute to the metabolites of L-DOPA acetoacetate in the same manner as L-DOPA. And the following three possibilities are supposed. 1) The possibility which L-DOPA acetoacetate can be decomposed into L-DOPA and acetoacetate before the uptake into nerve ending or NE store and which is converted to CA after the uptake in the form of L-DOPA. 2) The possibility which L-DOPA acetoacetate can be decomposed into L-DOPA and acetoacetate after the uptake into nerve ending or NE store and which is converted to CA turning into L-DOPA. 3) The possibility which L-DOPA acetoacetate is converted to acetoacetate of CA in nerve ending or NE store, so-called acting as a false transmitter, and/or which acts in the mixed form with 2). Since it is not clear from the results of these experiments whether any form among them has influence on the response, we must leave this for a future study. However, since the tyramine-induced tachyphylaxis was not restored by the pretreatment of a peripheral DOPA decarboxylase inhibitor, Ro4-4602, it could be comprehended that the restoration was not due to L-DOPA acetoacetate itself but the metabolites of L-DOPA acetoacetate.

Furthermore, the tachyphylaxis induced by the direct action of L-DOPA acetoacetate or L-DOPA is supposed as a possibility. Judging from the fact that the contractions of aorta and vas deferens induced by CAs were not affected by both compounds, however, it could be objected to the direct action.

In the isolated smooth muscle preparations, after the tyramine-induced tachyphylaxis and the abolishment of the response of tyramine after reserpine pretreatment, these contractions were restored by L-DOPA acetoacetate and L-DOPA. But the effects of both compounds on contractile responses induced by other agonists were not observed. In the isolated rat uterus, as an exception, the spontaneous motility was increased by either compound and the contractions induced by ACh and oxytocin were potentiated. It is considered that these actions are due to either an increase of the ion permeability of membrane or to an increase of the sensitivity of muscle itself, but that both compounds affect selectively on uterus setting aside the mechanism because they have no effect on other smooth muscles except uterus. The results of our experiments in the rat uterus contradicted with those of Kasahara, *et al.*[10]

14) H. Takashima, *Nippon Yakugaku Zasshi*, **58**, 437 (1962).

in which the spontaneous motility in diestrus rat uterus was slightly decreased by L-DOPA.

The effects of L-DOPA acetoacetate and L-DOPA on the gastrointestinal propulsion in mice and on the isolated skeletal muscle preparations in frog were not observed.

On the basis of the present knowledge, it could be concluded that L-DOPA possessed both of central and peripheral actions, whereas L-DOPA acetoacetate possessed only peripheral without central action since either it cannot pass the blood-brain-barrier or has not the action even if it was able to pass and that the activities would be due to their metabolites.

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