Pharmacological Studies on Diphenylalkylamine Derivatives. (I)*

Comparative Studies of the Actions of the Optical Isomers of 1,2-Diphenyl-1-dimethylaminoethane Hydrochloride

Hajime FUJIMURA and Kiyohisa KAWAI**

(Nakai Laboratory)

Received December 24, 1960

The phamacological effects of the two optical isomers of 1,2-diphenyl-1-dimethylaminoethane were comparatively studied.

A) Their effects were different in the following points: 1) The *l*-isomer showed a considerable analgesic effect not inferior to codeine phosphate, while the *d*-isomer showed no analgesic effect and even antagonized the same effects of morphine and tremorine. 2) Antitussive potency of the *l*-isomer was about one-fourth to one-half that of codeine; the *d*-isomer was also inert in this respect. 3) The rectal temperature of mice was decreased by the *l*-isomer, but increased by the *d*-isomer. 4) Both isomers inhibited the monoamine oxidase of the liver, however the effects of the *d*-isomer were greater than those of the *l*-isomer. 5) The *l*-isomer potentiated the hexabarbital sleeping time in mice, but the *d*-isomer did not.

B) Their effects were similar in the following points: 1) Toxic symptoms and LD 50 in mice. 2) Slight elevation of the blood sugar in rabbits. 3) Rise of blood pressure in the anesthetized cat and dog. 4) Antagonistic effect against the spasm of the extirpated intestines of rabbits and guinea-pigs induced by ACh, histamine and $BaCl_2$. 5) Constriction of the perfused ear vessels of the rabbit. 6) Local anesthetic effects on the rabbit's cornea and the guinea-pig's skin wheal were stronger than the effect of procaine.

From the above results, it is concluded that the *l*-isomer would be useful as a clinical analgesic agent.

INTRODUCTION

Although morphine has a variety of pharmacological actions, its characteristically powerful analgesic action is not to be considered as derived from its action on a pain center (in a narrow sense) but from the summation of its many actions on various sites in the body. On the other hand, morphine has many unpleasant side effects. It is widely believed that simplifying or partially modifying the structure of morphine could differentiate or separate some of its many varied actions. On the basis of this hypothesis the alkylamine portion which links the A and C rings of the morphine structure has been simplified. It has been considered that diphenylamine derivatives possess the minimal structural requirements for analgesic action. A large number of these derivatives have been studied and reported in the litera-

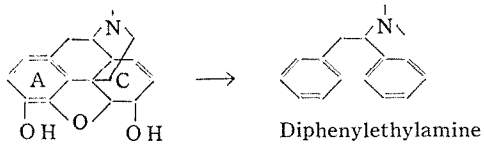
* The original paper of this report was written in Japanese and appeared in *Folia pharmacol. Japan*, 56, 514 (1960).

** 藤村 一, 川井清尚

ture but only a few papers have appeared concerning the weak analgesic effect which these compounds exert in animals[1]. In the previous reports the racemic compounds of these drugs were mainly studied. Not only morphine but also many synthetic analgesics are optically active. The previous studies of a number of these derivatives and their isomers prepared in this laboratory revealed that the tertiary amine derivatives and the levo-rotatory isomers were most effective in the screening test for analgesia and toxicity[2]. In this report the pharmacological actions of 1,2-diphenylaminoethane hydrochloride are further studied in an endeavor to obtain detailed information concerning the structure-activity relationship between morphine and phenylisopropylamine compounds, and to obtain more information of the relationship between optical activity and pharmacological action.

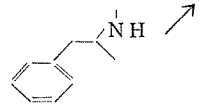
The melting points and specific rotations of the optical isomers of 1, 2-diphenyl-1-dimethylaminoethane hydrochloride are given below.

	M.P., °C	$[\alpha]_D^{20}(H_2O)$
Levo-Compound	218–219	−91.7
Dextro-Compound	218–219	+90.3
Racemic-Compound	187 and 210–211 (double melting point)	



Morphine ring

Diphenylethylamine



Amphetamine ring

EXPERIMENTAL RESULTS

1. Toxicity

The intraperitoneal injection of small doses of the isomers in mice revealed sedation, but in doses above 50 mg/kg the procedure revealed tonic and clonic convulsions, lying-down, inactivity and dyspnea followed by death. The patterns of the toxic symptoms of both optical isomers were not significantly different. As shown in Table 1, the LD 50 of the isomers in mice were also not significantly different. The body-weight-gain curves of young male rats were investigated. The

Table 1. Acute toxicity and analgesic actions by Haffner methods.

Isomers	LD50 mg./10 g., i. p. (95% limits)	ED50 mg./10 g., i. p. (95% limits)	
		Single	Threshold dose Morphine added
l-	1.30 (1.20-1.41)	0.35 (0.25-0.48)	0.11 (0.07-0.18)
d-	1.45 (1.32-1.58)	0	0.5mg.—Ca 50%
racemi-	1.23 (1.02-1.49)	0.5mg.—Ca 20%	0.16 (0.13-0.21)

average body weight of the rats was 55±5 g. A group of 20 animals was used for each isomer, and intraperitoneal injections of 5 mg/kg of each isomer, daily for 30 days, did not cause any significant difference from control animals. During this period the animals did not show any pathological signs (Fig. 1).

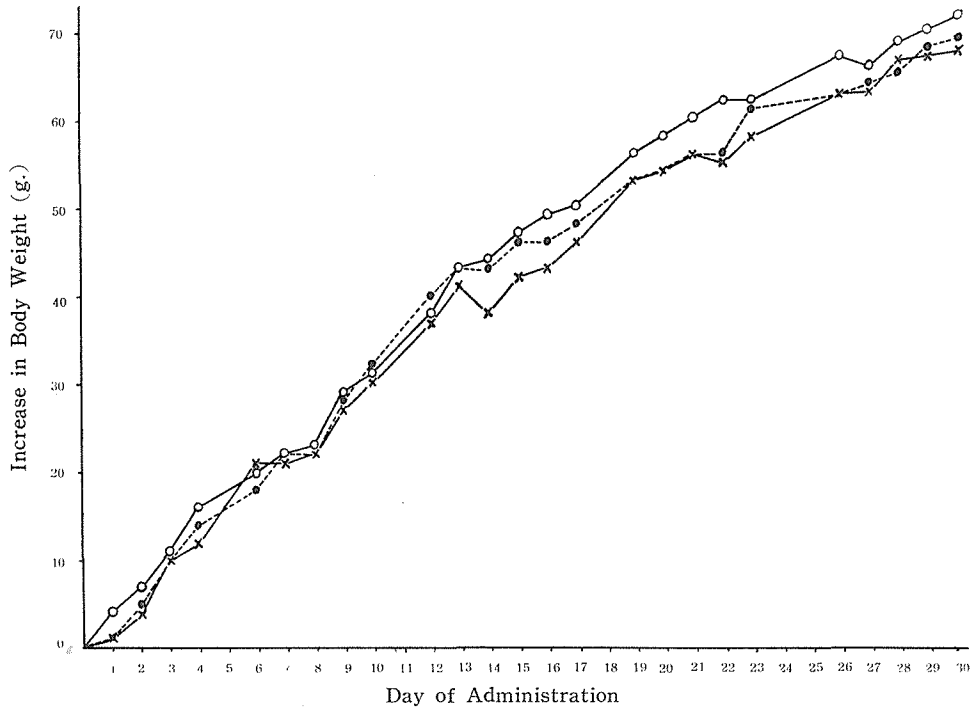


Fig. 1. Body weight gain curve of rats during the prolonged administration. —×—×— Control; —○—○— *l*-isomer, dose 5mg/kg.; ···●···●··· *d*-isomer, dose5mg/kg.; Average body weight at the start 55±5g. (One group includes 20 animals)

2. Analgesic Action

a) Haffner method. By this method the ED 50 of the *l*-isomer was obtained. The d-isomer did not show any analgesic activity, while the racemic compounds showed about 20% activity in a dose of 50 mg/kg. When the threshold dose of morphine for analgesic action (5 mg/kg subcutaneously) was injected in combination with one of the isomers, the most powerful analgesic effect was obtained with the *l*-isomer, and also considerable analgesic effect was obtained with the racemic compound. The *d*-isomer showed only about 50 % activity when a dose of 50 mg/kg was combined with morphine (Table 1).

b) D'Amour-Smith method. In this method prolongation of the reaction time above 15 seconds was regarded as positive analgesia produced by the drug. In subcutaneous doses of 50 mg/kg of the drug, the animals showed 55% analgesia for the *l*-isomer and 10% for the racemic compound respectively, but no effect for the *d*-isomer.

c) Synergism and antagonism of the drug on the analgesic effect of morphine

or tremorine[3]. Using the Haffner method the effects of the drug on the analgesic action of morphine or tremorine were studied. The *l*-isomer always showed a synergistic effect of the additive type on the analgesic sction of morphine or tremorine, whereas the *d*-isomer showed an antagonistic effect on the analgesic action of morphine or tremorine. As is shown in Table 2 the analgesic action of tremorine was blocked by the use of the *d*-isomer. It was interesting to note that tremor and some parasympathomimetic signs induced by tremorine were not affected.

Table 2. Synergism and antagonism by combination with morphine and tremorine in analgesic action. (Haffner Method)

Morphine, mg./10g. subcutaneously	Levo-rotating isomer, combined with 0.4mg./10g	Dextro-rotating isomer, combined with 0.4mg./10g.
0.05	60%	0%
0.10	80	50
0.15	100	80
0.20	—	50
Tremorine, mg./10g. subcutaneously		
0.05	40	0
0.10	60	0
0.15	(0.2mg.—60%)	(0.2mg.—30%)
0.15	80	0
0.20	90	0

In the table, % shows positivity in analgesic action in experimental animal whose 1 group includes 10 animals.

3. Synergistic Effect on the Sleeping Time of Methylhexabarbital Sodium (MHA)

The effect of the isomers on the sleeping time of MHA were tested in mice. Disappearance of the righting reflex in animals which received MHA or MHA and one of the isomers was regarded as an indication of sleeping. Both isomers, in a dose of 20 mg/kg, had no effect on the sleeping time of mice which received MHA in a dose of 80 mg/kg. But in a dose of 50 mg/kg, only the *l*-isomer clearly prolonged the sleeping time (Table 3). When 25 mg/kg of MHA, which otherwise was ineffective, was administered in combination with 50 mg/kg of the *l*-isomer, the

Table 3. Synergism with the sleeping action of methylhexabital (mice).

Isomers, dose combined, mg/10g, intraperitoneally		MHA, dose, mg/10g, i.p.	Average sleeping time (min.) ± S.E.	Prolongation rate
Control		0.8	123±13.8	1.00
Levo,	0.2	0.8	137±17.8	1.11
	0.5	0.8	205±23.1	1.66
Control		0.8	127±17.3	1.00
Dextro,	0.2	0.8	137±20.5	1.08
	0.5	0.8	163±17.7	1.28

animals lost their righting reflex for ninety minutes, while the animals which received the same doses of MHA and the *d*-isomer did not.

4. Influence on Body Temperature

The rectal temperature of the mice showed a steady fall in response to 40mg/kg of the *l*-isomer, and the maximal fall in temperature was obtained ninety minutes after injection. On the other hand, the rectal temperature revealed a slight elevation in response to the same dose of the *d*-isomer. The racemic compound had no effect on the rectal temperature of mice (Table 4).

Table 4. Influence of the isomers on the body temperature in mice.

Isomers, dose administered, 0.4mg/10g	Body temp. before administration °C±S. E.	Rectal temperature after		
		30 min.	60 min.	120 min.
levo-	38.2±0.09	37.2±0.19	36.6±0.16	37.1±0.23
dextro-	38.1±0.10	38.9±0.16	38.8±0.21	38.4±0.18
racemi-	38.2±0.06	38.4±0.11	38.0±0.14	38.0±0.21

5. Anti-tussive Action

Fujimoto[4] in this laboratory assayed the anti-tussive potency of the *l*-isomer in the dog. Cough was produced by electrical, mechanical or chemical stimulation of the trachea, and the restlting change of the abdominal respiratory pressure was recorded on smoked paper. The minimal effective dose of the *l*-isomer to suppress the cough (4 to 8 mg/kg intraveneously) was twice that of codeine. The *d*-isomer showed hardly any suppressing effect on the electrically induced cough in a dose below 10 mg/kg.

6. Effects on Monoamine Oxidase and Respiration of Brain Tissue

Previous studies[5] on a number of drugs revealed that no pararellism existed between central stimulating action *in vivo* and the inhibitory action on monoamine oxidase of rabbit's liver or the inhibitory action on respiration of rabbit's brain tissue *in vitro*. The inhibitory action of the *d*-isomer and the racemic compound of this drug on monoamine oxidase of the liver was twice as strong as that of amphetamine, whereas the *l*-isomer was much weaker in this respect. The *d*-isomer also showed a stronger inhibitory effect on respiration of rabbit cerebral tissue than did the *l*-isomer (Table 5, 1 and 2).

Table 5, 1. Influence on the oxidation of tyramine ($2\times10^{-3}M$) by monoamine oxidase in rabbit liver.

Dose		O_2 consumption (μL)	Inhibition (%)
Control		25.0±0.98	
Dextro-	$2\times10^{-3}M$	6.1±0.53	75.6
Levo-	〃	17.9±0.51	28.4
Racemi-	〃	9.9±0.55	60.4

Table 5, 2. Influence on the respiration of the cerebral cortical tissues of rabbit.

Dose		Qo_2	Dose	Qo_2	Inhibition (%) $2\times10^{-3}M$	$5\times10^{-3}M$
Control		9.3±0.19		10.0±0.41		
Dextro-	$2\times10^{-3}M$	8.2±0.25	$5\times10^{-3}M$	6.7±0.38	11.8	33.0
Levo-	〃	9.1±0.13	〃	7.7±0.36	2.1	23.0
Racemi-	〃	9.0±0.49	〃	7.1±0.35	3.2	29.0

7. Effect on Blood Glucose in Rabbits

The effect of the intravenous injection of 5 mg/kg of the drug on the blood glucose level was studied in rabbits by using the Somogyi method. Both the *l*- and *d*-isomers slightly raised the blood glucose level. Peak effects were obtained about sixty minutes after administration of these drugs. In this dose there was no significant difference in the activity of the two isomers. The administration of the same dose of morphine increased blood sugar about 60%. In this case, the peak effect was obtained about 120 minutes after administration (Table 6).

Table 6. Increase of the blood sugar level in rabbit (Somogyi method).

Isomers	Increase after administration				
	30min	60	120	180	240
Levo-	16±4.8	18±6.0	11±7.2	8±6.7	6±3.5
Dextro-	9±6.9	16±7.1	14±6.3	13±5.8	9±4.8

The values are average increases in % ± S. E. by intravenous injection of 5mg./kg. dose.

8. Effects on Blood Pressure in Dogs and Cats

The effects of the isomers on carotid arterial blood pressure were studied in dogs weighing 5 to 10 kg and cats weighing 2 to 4 kg anesthetized with 50 to 60 mg/kg of amytal sodium. In dogs 1 mg/kg of both isomers injected intravenously caused a slight and persistent rise in blood pressure (10 to 20 mm Hg). In no case was a fall in blood pressure observed. Repeated administration of the drugs in the same dose did not give rise to tachyphylaxis. A similar rise of blood pressure in response to the isomers was also observed in cats. However, intravenous injection of 1 mg/kg of either isomer to cats, in which the spinal cord had been previously sectioned between CI and CII under ether anesthesia, revealed a transient fall in blood pressure about 30 mm Hg (Figs. 2, 3 and 4).

9. Effect on the Perfused Ear Vessels of Rabbits

The isolated ear vessels of a rabbit were perfused with Ringer's solution according to the method of Pissemski, and the test solution of the isomers was injected into the rubber tube leading to the perfusion cannula. The administration of both isomers in a dose of 1 mg constricted the blood vessels (Fig. 5).

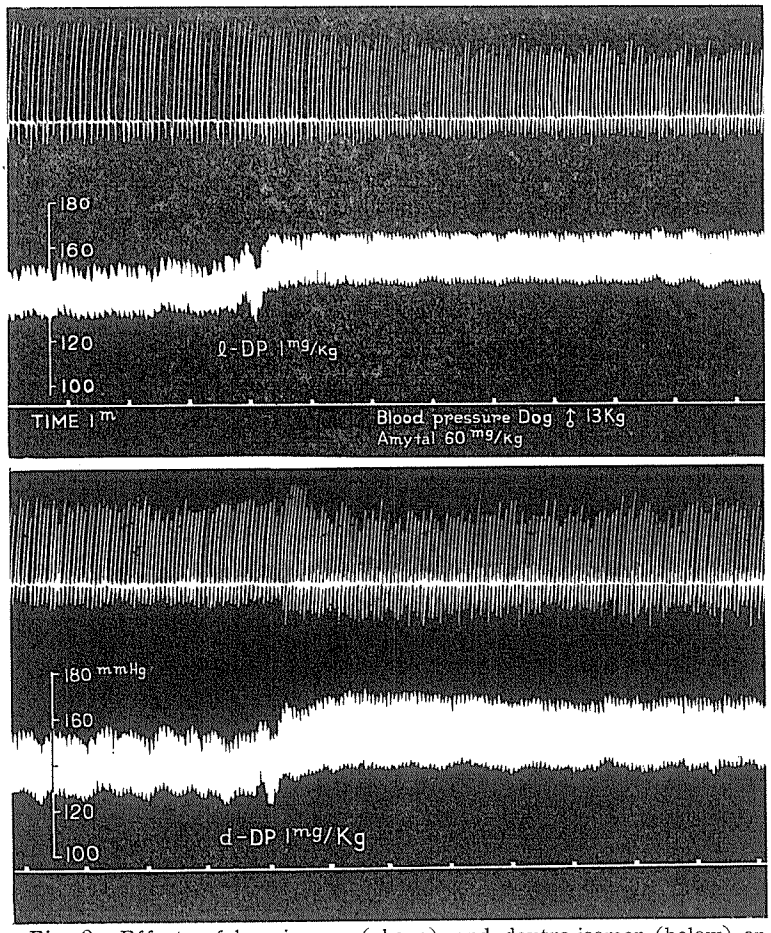
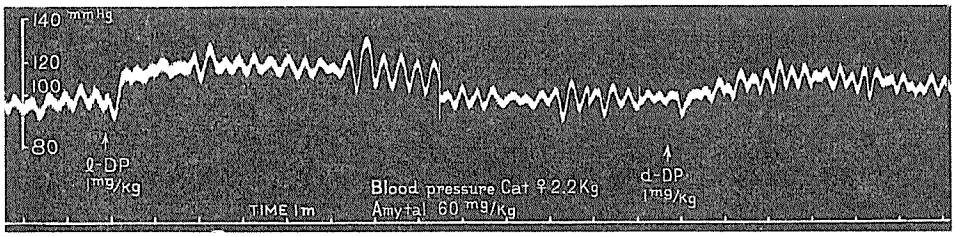


Fig. 2. Effects of levo-isomer (above) and dextro-isomer (below) on the blood pressure and respiration in dogs.



↑ *l*-Isomer ↑ *d*-Isomer

Fig. 3. Effects of the isomers on the blood pressure in cats.

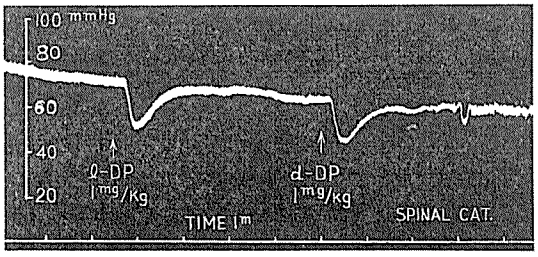


Fig. 4. Effects of the isomers on the blood pressure in spinal cat.

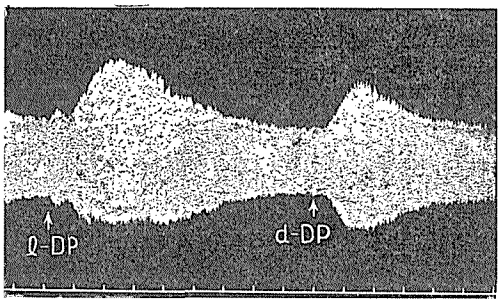


Fig. 5. Effects of the isomers on the rabbit ear vein.

10. Antispasmodic Action on the Isolated Intestine and Tracheal Muscle

The application of the isomers inhibited the contraction of the isolated rabbit ileum induced by 10^{-7} of acetylcholine, 10^{-7} of histamine and 10^{-4} of $BaCl_2$; and also the contraction of the isolated guinea-pig ileum induced by 10^{-7} of histamine. The minimal inhibitory dose of the isomers was 5×10^{-5} to $2\sim4\times10^{-6}$. Contraction of the ring preparation of guinea-pig trachea induced by 10^{-6} of ACh and 10^{-6} of histamine, and also contraction of the same preparation of rabbit trachea induced by 10^{-6} of ACh were also inhibited almost to the same degree by application of each isomer. As is shown in Tables 7 and 8, the antagonistic effects of the isomers on the spasm of smooth muscle were always weaker than similar effects of atropine, benadryl and papaverine.

Table 7. Antispasmodic action on the isolated intestine (minimum inhibiting concentration).

Isomers	Anti-acetylcholine (1×10^{-7})	Anti-histamine* (1×10^{-7})	Anti-$BaCl_2$ (1×10^{-4})
Levo-	$2\sim4\times10^{-6}$	$2\sim4\times10^{-5}$	5×10^{-5}
Dextro-	$2\sim4\times10^{-6}$	5×10^{-5}	1×10^{-5}
Racemi-	$2\sim4\times10^{-6}$	5×10^{-5}	5×10^{-5}

* Guinea pig intestine; other data on rabbit intestine.

Table 8. Antispasmodic action on the isolated tracheal muscle (minimum inhibiting concentration).

Isomers	Anti-acetylcholine (1×10^{-6})	Anti-histamine (1×10^{-6})	Anti-acetylcholine* (1×10^{-6})
Levo-	$1\sim5\times10^{-5}$	1×10^{-5}	1×10^{-5}
Dextro-	1×10^{-5}	1×10^{-5}	2×10^{-6}
Racemi-	$1\sim5\times10^{-5}$	1×10^{-5}	2×10^{-6}

* Rabbit muscle; other data on guinea pig muscle.

11. Local Anesthetic Action

a) Surface anesthesia 0.1 ml of a 1% solution of the isomers was placed on the cornea of the intact rabbit and the central portion of the cornea was stimulated with a piece of brush hair five times every five minutes after drug application.

When the corneal reflex did not respond to at least 3 of the 5 stimuli, it was regarded as a positive anesthesia and the duration of the anesthesia was thus determined. The average number of positive responses to each isomer in five animals did not show a marked difference in the anesthetic action of the two isomers. On the whole, the local anesthetic action of the isomers was longer in duration than that of procaine. Namely, the duration of action after the application of a 1% solution was 10±1.6 minutes for procaine, 15±2.9 minutes for the *l*-isomer and 14 ±1.3 for the *d*-isomer.

b) Infiltration anesthesia. According to the method of Bülbring and Wajda[6], 0.2 ml of various concentrations of the isomers were injected intracutaneously in the back of guinea-pigs. The animals were used as a group for one dose. The injection site was stimulated with a needle six times every five minutes until a twitch response reappeared. The number of disappearances of the twitch responses was counted and plotted against the log. concentration of the isomers, as is shown in Fig. 6. The *l*-isomer was slightly stronger in anesthetic action than the *d*-isomer. The local anesthetic action of either isomer was stronger than that of procaine.

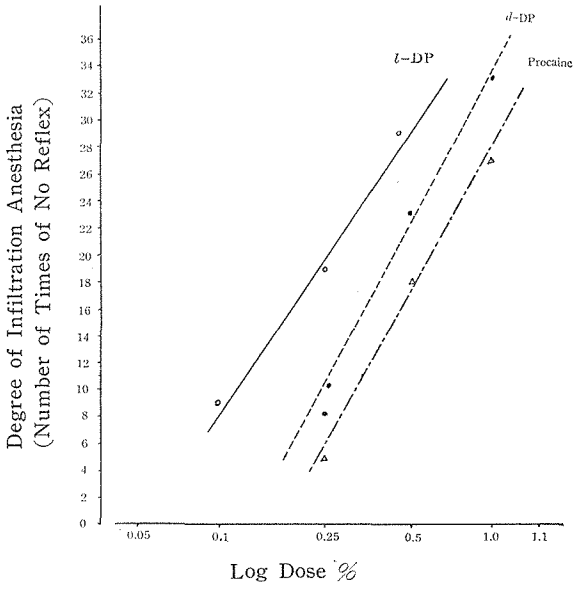


Fig. 6. Infiltrating anesthetizing action of the isomers (intracutaneously injected at back of guinea pigs).
l-DP=levo-isomer, *d*-DP=dextro-isomer

DISCUSSION AND SUMMARY

From the results mentioned above, some differences of the pharmacological actions between the *l*- and *d*-isomers of 1, 2-diphenyl-l-dimethylaminoethane are cited as follows: 1) Analgesic action was obtained only by the *l*-isomer, but not by the *d*-isomer. The potency of the action of the former was equivalent to that of codeine in the Haffner method, but was too weak to be evaluated by the D'Amour-Smith method. The analgesic actions of morphine and tremorine were potentiated by the *l*-isomer, but the same actions were antagonized by the *d*-isomer. These results may

be clarified by noting that the racemic compound of this drug was much weaker in its analgesic action and that the evaluation of its potency (ED 50) was difficult in the Haffner method in which the threshold dose of morphine was administered. 2) The anti-tussive effect of the *l*-isomer in dogs was equivalent in potency to one-fourth to one-half of that of codeine. The *d*-isomer did not show such an effect. 3) The sleeping time of mice produced by methylhexabarbital sodium was prolonged by the *l*-isomer, but not by the *d*-isomer. 4) The *l*-isomer lowered the rectal temperature of mice, but the *d*-isomer raised it. Similar sequences were also obtained by the optical isomers of phenyl-isopropylamine. 5) Both isomers had an inhibitory action on the monoamine oxidase of rabbit liver. The *d*-isomer was even stronger in its action than *d*-amphetamine. These results suggest that there is no sign of pararellism between the analgesic and the central stimulating activity of these isomers, and the inhibitory action on monoamine oxidase activity.

Some similarities in the pharmacological actions of the two isomers were as follows: 1) The toxicity studies in mice showed that small doses of the isomers caused sedation, but large doses caused convulsion followed by fatal respiratory paralysis. The isomers never caused an increase to spontaneous motility as did amphetamine or morphine. No significant difference was observed in the LD 50 of these isomers. 2) The blood glucose level was slightly elevated in response to both isomers. It is generally reported of the rabbit that the extent of the elevation of blood glucose by analgesic drugs pararells the potency of the drug's analgesic action[7]. The fact that the *d*-isomer of this drug raised the blood glucose in spite of its non-analgesic property, offers evidence against this hypothesis. 3) Both isomers slightly but persistently increased the blood pressure in dogs and cats. This is a sharp contrast to the action of morphine which persistently lowers blood pressure. 4) The inhibitory action of the *l*-isomer on the spasm of smooth muscle induced by acetylcholine, histamine and $BaCl_2$ was almost of the same order as that of the *d*-isomer. This inhibition was not specific. 5) The isomers constricted the perfused isolated ear vessels of the rabbit. 6) The local anesthetic potency of both isomers was of the same order, but was always greater than that of procaine.

The summary and discussion of the actions of both isomers, cited above, clearly show that they distinctly differed in their central action, but that they have many similarities in their peripheral effects.

The evidence that the *l*-isomer has many different actions from morphine and amphetamine, to which it is closely related in chemical structure, has provided the subject for the following detailed pharmacological studies.

The authers experess their sincerest gratitude to Professor Emeritus K. Ogiu for his kind guidance and encouragement.

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