PHARMACOLOGICAL STUDIES ON DL-PIPERONYL-METHYLAMINOPROPANE

I PERIPHERAL ACTION

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INTRODUCTION

Among the adrenergic drugs, the excitation effect of ephedrine and the phenylalkylamines (amphetamine and methamphetamine) on the adrenergic nerves and the central nervous system is well known. The inhibition of monoamine oxidase has been supported since Gaddum et al. (1938) as the mechanism for these actions. However, based on the subsequent experiments on the changes in action of adrenergic drugs through adrenergic nerve denervation and cocaine administration (Fleckenstein et al. 1953) and experiments using reserpine (Burn et al. 1958), adrenergic drugs were classified into 3 groups (Trendelenburg et al. 1962). 1) those with a direct action on the receptors (catecholamines-direct acting amines), 2) those with an indirect action on the receptors through the release of catecholamines especially norepinephrine from the adrenergic nerve terminal (tyramine and amphetamine-like drugs-indirect acting amines), 3) those with both of these actions (ephedrine-like drugs-mixed acting amines).

Based on these facts, Takasaki (1966, a, c) compared the intensity of the hypertensive effect with reserpine administered after ephedrine and methamphetamine, and discussed about the mechanism of action on the release of norepinephrine from the adrenergic nerve storage sites.

On the other hand, studies were also conducted on drugs obtained through changes of the chemical structure of phenylalkylamines (Hauschild, 1940., Marsh et al. 1950., Matsushima, 1957).

Recently, Mizunoe (1961) reported on the actions of the *d* and *l* isomers of piperonyl-methylaminopropane produced through modification of the phenyl ring of methamphetamine. However, they failed to clarify the mechanism of the action described above.

In the present experiment, *dl* isomer of piperonyl-methylaminopropane was used to study the effect of introducing a piperonyl group on its action, including the basic mechanism of action.

MATERIALS AND METHODS

1. Chemical structure

dl-Piperonyl-methylaminopropane (*dl*-PMAP) has the following chemical structure with the chemical name of 1-piperonyl-2-methylaminopropane, with substitution for the phenyl group of methamphetamine by a piperonyl group. *dl*-PMAP hydrochloride was used in this experiment.

m. p. = 179.5 °C

R	Name
Phenyl-methylaminopropane (Methamphetamine)
Piperonyl-methylaminopropane

As for other drugs, *d*-methamphetamine (methamphetamine hydrochloride, Dainihon Seiyaku Co.), reserpine (Triserpin, Torii Seiyaku Co.), cocaine (cocaine hydrochloride, Sankyo Seiyaku Co.), tolazoline (Imidaline, 2-benzyl imidazoline hydrochloride, Yamanouchi Seiyaku Co.) and *dl*-norepinephrine (norepinephrine bitartrate, Sankyo Seiyaku Co.) were also employed in this experiment.

2. Observation on effects with intravenous administration in anesthetized animals.

The tested drugs were diluted or dissolved in physiological saline solution and injected into the femoral vein.

Dog

At 30 min after subcutaneous injection of 10 mg/kg of morphine, 15 mg/kg of pentobarbital sodium was intravenously injected for anesthesia. In some experiments, 1 mg/kg of atropine was subcutaneously injected along with the morphine.

Respiratory movements were recorded through a cannula introduced into the trachea connected to a tambour via a rubber tube.

Blood pressure changes were recorded by means of a cannula filled with heparin solution inserted into the common carotid artery, connected with a mercury manometer via a rubber tube filled with saline solution, and simultaneously recorded on a smoked drum.

In some dogs, the drug was administered into the common carotid artery of the opposite side via the superior thyroid artery.

In still other experiments, a polygraph (Model RM 150 M, Nihon Koden Kogyo Co.) was used to record the femoral arterial pressure with the aid of a transducer and to lead a II electrocardiogram (ECG) simultaneously.

Rabbit

A rabbit injected subcutaneously with 1g/kg of urethane was used. The changes in intestinal motility were recorded in vivo with the Trendelenburg method.

In other experiments, a cardiotaubour of the Nuki type (1962) was used to record the cardiac action of rabbit in vivo on smoked paper on a kymographion.

3. Observation on the isolated organs.

dl-PMAP was dissolved or diluted (g/ml) with the nutritional solution in each experiment. Unless otherwise specified, a kymographion was used to record on a smoked paper.

a) Heart

Using Ringer solution for cold blooded animals as the nutritional solution, the action of an isolated frog heart was studied by the Straub method. The Langendorff method was used to perfuse the isolated rabbit heart with Locke solution (36~37°C, 60~80 mmHg).

b) Intestine

With the Magnus method, changes in the motility of the isolated rabbit small intestines were recorded. The drug solution was added in a constant amount to the isolated tissue bath containing Tyrode solution.

c) Blood vessel

The Kraukow-Pissemsky method was used in perfusing the auricular artery of rabbits and the drug was added to the perfusate (Ringer solution for warm blooded animals, 36~37°C) in adjusting to a dose of 0.5 ml.

The effect of the drug on the whole frog vessels as described by Fukuda (1927) was tested, using Ringer solution for cold blooded animals at a pressure of approximately 20 cm H₂O. The dose of drug was adjusted to 0.5 ml to be injected into the rubber tube before cannulation of the blood vessel.

On the other hand, a digital artery of the dog paw was perfused with heparinized blood under constant pressure as reported by Takasaki (1966 b). The drug dissolved with saline solution, 0.02 ml within 10 sec, was administered in injection area before the arterial cannulation. A drop recorder was used for recording and the changes in inflow were recorded on polygraph recording paper. A sigmamotor pump (Model TW-12, Middeport, N. Y., U. S. A.) was used to perfuse a certain flow rate of heparinized blood and the changes in blood pressure through the administration of the drug solution were recorded.

Further, experiments were carried out with the isolated artery. Rabbits were thoracotomized after bleeding to remove the aortic arch, and the common

carotid artery was removed from the anesthetized dog. These arteries were cut spirally 2-2.5 mm in width and 2 cm in length and the contraction was recorded by the Magnus method.

d) Spleen

Rabbits were sacrificed by bleeding and laparotomized. After splenectomy, 2 cm of the organ was resected and the contracture was recorded by the Magnus method. The nutritional solution, a Tyrode solution, was used. The writing lever was about 0.5 g in weight.

RESULTS

1) Effect on respiration and blood pressure of dogs.

Respiration was frequently inhibited immediately after the intravenous administration of 0.1-5 mg/kg of *dl*-PMAP temporarily, followed by slight excitation in a few instances.

According to the dose, blood pressure tended to rise immediately after *dl*-PMAP administration. In some animals, a mild depression of blood pressure preceded the rise. The intravenous administration of 1 mg/kg of reserpine after the return of blood pressure almost to the normal level after *dl*-PMAP administration caused a marked rise in blood pressure lasting 1 hr or more.

Fig. 1 The average changes of blood pressure rise according to time in percentage following the administration of reserpine 1 mg/kg after *dl*-PMAP in comparison with methamphetamine in dogs. •—• *dl*-PMAP 0.5 mg/kg (3 dogs). •··• Methamphetamine 0.5 mg/kg (4 dogs) (This result was obtained in the previous experiment (Takasaki, 1966 a)). Arrow shows the injection. The abscissa is the time in minutes and the ordinate is the percentage of pressure.

However, the single administration of 1 mg/kg of reserpine never caused a rise in blood pressure and only a drop in blood pressure occurred. Pretreatment with *dl*-PMAP resulted in a pressor effect of reserpine. Fig. 1 depicts the chronological changes in the average blood pressure rise following the administration of 1 mg/kg of reserpine after *dl*-PMAP, in comparison with methamphetamine. When *dl*-PMAP in a dose the same as the initial dose was administered 1 hr after reserpine, a more pronounced pressor effect was elicited. When no *dl*-PMAP was administered initially and reserpine was injected initially followed by *dl*-PMAP 1 hr later, the blood pressure rise was even more

Fig. 2 The blood pressure changes caused by *dl*-PMAP following the administration of reserpine in dogs. Upper tracing: Dog A. Reserpine 1 mg/kg alone. Middle tracing: Dog B. Left- *dl*-PMAP 0.5 mg/kg. Middle- Reserpine 1 mg/kg after *dl*-PMAP 0.5 mg/kg. Right- *dl*-PMAP 0.5 mg/kg 1 hr after reserpine 1 mg/kg. Lower tracing: Left- Dog C. *dl*-PMAP 0.5 mg/kg 24 hr after reserpine 1 mg/kg. Middle- Dog D. Norepinephrine 3 µg/kg. Right- Dog E. Norepinephrine 1 µg/kg 24 hr after reserpine 1 mg/kg. The respiratory movement was also recorded in middle and lower tracings. Drugs were injected at the arrow. Scale is in mmHg. Time interval is 10 seconds. Remarks are the same as those of Fig. 4, 5, 6, 8 and 9.

Fig. 3 The dose response curve for pressor effects of *dl*-PMAP in unreserpinized and reserpinized dogs. $\circ$ — $\circ$ Unreserpinized dog. $\bullet$ — $\bullet$ Reserpinized dog (24 hr). The abscissa shows the administered dose of the drug in logarithmic scale of mg/kg and the ordinate shows a scale in mmHg. Vertical bar represents the standard error (one side) on 3 to 4 experiments.

Fig. 4 The pressor effect of *dl*-PMAP on dogs after the intravenous administration of cocaine. Left- Control. *dl*-PMAP 0.5 mg/kg. Middle- *dl*-PMAP 0.5 mg/kg 1 hr after reserpine 1 mg/kg. Right- *dl*-PMAP 0.5 mg/kg after cocaine 5 mg/kg.

Fig. 5 The pressor effect of *dl*-PMAP on dogs after the intravenous administration of tolazoline. Left- Control. *dl*-PMAP 0.5 mg/kg. Middle- *dl*-PMAP 0.5 mg/kg 1 hr after reserpine 1 mg/kg. Right- *dl*-PMAP 0.5 mg/kg after tolazoline 10 mg/kg.

pronounced than in animals in which *dl*-PMAP was administered before reserpine. On the other hand, when reserpine 1 mg/kg was subcutaneously injected followed by intravenous injection of *dl*-PMAP 24 hr later, the rise was markedly decreased. However, the pressor effect of norepinephrine was markedly potentiated as compared with normal animals. Fig. 2 shows these experimental results. Fig. 3 compares the dose response curve for pressor effects of *dl*-PMAP between reserpinized and unreserpinized animals. In dogs in which 1 mg/kg of atropine sulfate was subcutaneously injected along with morphine, a pressor effect of *dl*-PMAP occurred more markedly than in animals without atropine treatment. The pressor effect of *dl*-PMAP markedly reduced or even disappeared after the intravenous administration of 5 mg/kg of cocaine and 10 mg/kg of tolazoline (Fig. 4 and 5). The continuous rise of blood pressure in response to reserpine after *dl*-PMAP hardly occurred after the administration of cocaine or tolazoline. The administration of cocaine or tolazoline at the peak of the reserpine hypertension after *dl*-PMAP resulted in an immediate reducing or disappearance of the pressor effect.

On the other hand, a mild depression of blood pressure immediately after *dl*-PMAP administration lessened or disappeared after bilateral vagotomy or the intravenous administration of 1 mg/kg of atropine (Fig. 6). Fig. 7 compared the pressor effects after the administration of 0.5 mg/kg of *dl*-PMAP with that after administration of 0.2 or 0.5 mg/kg of methamphetamine and 2 μ g/kg of norepinephrine in atropinized dogs.

At the second administration of 0.5 mg/kg of methamphetamine, a marked weakening of the pressor effect (so-called tachyphylaxis) frequently occurred. Tachyphylaxis was not found until after several attempts at repeated administration and not readily induced at a dose of *dl*-PMAP less than 0.5 mg/kg. The administration of more than 1 mg/kg of *dl*-PMAP as frequent as that of 0.5 mg/kg of methamphetamine definitely resulted in tachyphylaxis. When 1 mg/kg of *dl*-PMAP was repeatedly injected intravenously at intervals of about 15 min, the pressor effect gradually declined until it disappeared on the 4th-5th injection and a depressor effect was seen instead. However, the administration

Fig. 6 The effect of bilateral vagotomy or the intravenous administration of atropine on dogs. Left- Control. *dl*-PMAP 1 mg/kg. Middle- *dl*-PMAP 1 mg/kg after bilateral vagotomy. Right- *dl*-PMAP 1 mg/kg after atropine 1 mg/kg.

Fig. 7 Comparison of the pressor effects after the administration of *dl*-PMAP with those of methamphetamine and norepinephrine after the administration of reserpine and cocaine in atropinized dogs. The ordinate is the percentage of pressure. Vertical bar shows the standard error (one side) on 3 to 5 experiments. From left to right. First column: *dl*-PMAP 0.5 mg/kg. [] Control. [] *dl*-PMAP 1 hr after reserpine 1 mg/kg. [] *dl*-PMAP 24 hr after reserpine 1 mg/kg. [] *dl*-PMAP after cocaine 5 mg/kg. Second column: Methamphetamine 0.2 mg/kg. [] Control. [] Methamphetamine 1 hr after reserpine 1 mg/kg. [] Methamphetamine 24 hr after reserpine 1 mg/kg. Third column: Methamphetamine 0.5 mg/kg. [] Control. [] Methamphetamine 1 hr after reserpine 1 mg/kg. [] Methamphetamine 24 hr after reserpine 1 mg/kg. [] Methamphetamine after cocaine 5 mg/kg. Fourth column: Norepinephrine 2 µg/kg. [] Control. [] Norepinephrine 1 hr after reserpine 1 mg/kg. [] Norepinephrine 24 hr after reserpine 1 mg/kg. [] Norepinephrine after cocaine 5 mg/kg.

of reserpine resulted in more difficulty in causing tachyphylaxis until the 3rd-4th injection. On the other hand, cross tachyphylaxis was observed between *dl*-PMAP and methamphetamine (Fig. 8).

Immediately after the injection of *dl*-PMAP 0.1-0.2 mg/kg into the common carotid artery, a transient, momentous respiratory excitation was found, followed by further mild excitation in some animals. The blood pressure then showed a gradual rise, lasting for a considerably longer period. After denervation of the carotid sinus nerve, the transient respiratory excitation disappeared but a subsequent mild excitation and pressor effect appeared without modification (Fig. 9).

Fig. 8 The cross tachyphylaxis between *dl*-PMAP and methamphetamine in dogs. Five figures from left show the repeated injection of *dl*-PMAP 1 mg/kg at intervals of about 15 min. Last figure is the result of methamphetamine 1 mg/kg after *dl*-PMAP tachyphylaxis.

Fig. 9 The effect on the respiration and blood pressure by *dl*-PMAP injected into common carotid artery of the dog. Upper tracing: *dl*-PMAP 0.2 mg/kg. Lower tracing: *dl*-PMAP 0.2 mg/kg after denervation of the carotid sinus nerve.

2) Action on the heart.

Fig. 10 shows the influence of *dl*-PMAP on the canine ECG. The intravenous administration of 1 mg/kg of *dl*-PMAP caused a rise of blood pressure and bradycardia. However, almost no change was recognized in the pattern of the ECG. Some slight augmentation of a T wave was seen at the most. After bilateral vagotomy, the administration of the same dose of *dl*-PMAP caused a simultaneous rise in blood pressure and a complete disappearance of bradycardia.

Scarcely any effect on cardiac motility was found *in vivo* upon administration of less than 1 mg/kg *dl*-PMAP into the vein of rabbits. The injection of 2 mg/kg caused a slight rise in the amplitude of cardiac movement along the longitudinal axis but no particular change was recognized along the transverse axis. Upon the administration of 5 mg/kg, a decrease of amplitude was observed in both the longitudinal and transverse axis (Fig. 11). Such a cardiac inhibitory effect was slightly decreased after bilateral vagotomy in some cases.

Fig. 10 The influence of *dl*-PMAP on the canine ECG and blood pressure. Upper two tracings: *dl*-PMAP 1 mg/kg. Lower two tracings: *dl*-PMAP after bilateral vagotomy. Each tracing shows the blood pressure (Upper record) and ECG (lower record). Scale is in mmHg. Time interval is 1 second.

Fig. 11 The effect of *dl*-PMAP administered intravenously on the cardiac motility of rabbits. Upper tracing: *dl*-PMAP 2 mg/kg. Lower tracing: *dl*-PMAP 5 mg/kg. Each tracing shows the cardiac movement of transverse and longitudinal axis from above. Time interval is 10 seconds.

Fig. 12 The effect of *dl*-PMAP on the isolated heart of frogs. Upper tracing: *dl*-PMAP 10⁻⁶. Middle tracing: *dl*-PMAP 10⁻⁴. Lower tracing: *dl*-PMAP 10⁻³. Time interval is 10 seconds.

Fig. 12 shows the effect of *dl*-PMAP on the isolated heart of frogs. Scarcely any change was seen at concentrations less than 10⁻⁸ of *dl*-PMAP, except for a mild increase in amplitude in a few cases. With the administration of 10⁻⁷ - 10⁻⁶ of *dl*-PMAP, a slight increase in amplitude was noted in many hearts, while a decrease of amplitude was found in some cases. In many hearts, both the amplitude and rate of contraction were inhibited by the administration of 10⁻⁵ of *dl*-PMAP. In some cases, a slight temporary rise in amplitude preceded this. At a concentration of 10⁻⁴, both the amplitude and cardiac rate were inhibited and diastolic standstill was recognized in many hearts, and an atrioventricular block occurred in a few cases. At concentrations above 10⁻³, the heart stopped in diastole immediately after administration.

The administration of methamphetamine gave a more intensive inhibitory effect on the amplitude at lower concentration than with the administration of *dl*-PMAP. A fall of the pulse rate and cardiac standstill also appeared at lower concentrations than with *dl*-PMAP in many hearts.

Fig. 13 shows the action of *dl*-PMAP on the isolated heart of rabbits. With the administration of 0.1 % of a *dl*-PMAP-Locke solution, 0.1 - 0.2 ml, the

Fig. 13 The effect of *dl*-PMAP on the isolated heart of rabbits. Upper tracing: *dl*-PMAP 0.1 % 0.2 ml. Lower tracing: *dl*-PMAP 0.1 % 0.5 ml. Time interval (—) is 10 seconds.

amplitude and cardiac rate were both increased and a systolic position was assumed in some hearts. The administration of 0.3 ml and over, however, caused a slight decrease in amplitude. The repeated administration of more than 0.1 ml caused a decrease in amplitude and cardiac rate after the second injection, depending upon the dosage used.

The administration of the same dose of methamphetamine gave almost the same effect as with *dl*-PMAP except for a slightly more intense excitation effect.

3) Action on the small intestinal tract.

After the intravenous administration of less than 1 mg/kg of *dl*-PMAP, scarcely any effect was found on rabbit intestinal motility. The administration of 2 mg/kg of *dl*-PMAP gave a slight inhibitory effect on amplitude immediately but 5 mg/kg caused a marked and sustained decrease in amplitude and a decreased motility rate of the intestine for a considerable period (Fig. 14).

Fig. 14 The effect of *dl*-PMAP, injected intravenously, on the motility of rabbit intestine. Upper tracing: *dl*-PMAP 2 mg/kg. Lower tracing: *dl*-PMAP 5 mg/kg. Time interval (|___|) is 10 seconds.

Fig. 15 shows the action of *dl*-PMAP on the isolated intestine of rabbits. At concentrations below 10^{-7} , scarcely any effect was noted. The administration of 10^{-6} - 10^{-5} caused a mild increase of amplitude or no change in some cases but a decrease of amplitude in many preparations. The administration of 10^{-4} definitely caused a decrease in amplitude and cardiac rate in many preparations. At concentrations above 10^{-3} , a marked decrease in amplitude and rate of

contraction was found in all cases. Especially at concentrations above 10^{-2} , immediate standstill occurred in many preparations.

The administration of the same dose of methamphetamine caused a more intense action than *dl*-PMAP and a definite decrease in amplitude was observed at 10^{-7} - 10^{-6} . At concentrations above 10^{-4} , temporary standstill of activity was recognized in many preparations. At concentrations of 10^{-3} - 10^{-2} , a marked decrease in amplitude and rate of motility were noted in all cases. Particularly at 10^{-2} , motor standstill was exhibited immediately after administration in many preparations. Administration of the same dose of methamphetamine gave a more intensive action than *dl*-PMAP. A definite decrease in amplitude was observed at concentrations of 10^{-7} - 10^{-6} . At concentrations of 10^{-4} and over, temporary motor standstill was exhibited in many preparations.

Fig. 15 The effect of *dl*-PMAP on the isolated rabbit intestine. Upper tracing : *dl*-PMAP 10^{-4} . Lower tracing : Methamphetamine 10^{-4} . Time interval is 10 seconds.

Fig. 16 The comparative effect of *dl*-PMAP and epinephrine by perfusion on the auricular artery of a rabbit ear. The abscissa shows the time in minutes and the ordinate is the number of drops. From upper illustration : *dl*-PMAP 10^{-4} - 10^{-3} (10 experiments), *dl*-PMAP 10^{-2} (3 experiments) and epinephrine 10^{-3} - 10^{-2} (4 experiments).

4) Effect on the blood vessels.

Fig. 16 compares the effect of *dl*-PMAP and epinephrine by perfusion on the auricular artery of a rabbit ear. At concentrations below 10^{-4} of *dl*-PMAP, almost no changes in flow were observed. At 10^{-3} , a mild vasoconstriction was found and the constriction became more definite at 10^{-2} , lasting for more than 30 min. Such an effect was somewhat weaker than with methamphetamine at the same concentration, being less than 1/5 that of epinephrine.

Fig. 17 The comparative effect of *dl*-PMAP and epinephrine by whole vessel perfusion on a frog body. The abscissa shows the time in minutes and the ordinate is the number of drops. Left illustration: *dl*-PMAP 10^{-7} - 10^{-4} (3 experiments), *dl*-PMAP 10^{-3} (5 experiments) and *dl*-PMAP 10^{-1} (3 experiments). Right illustration: Epinephrine 10^{-7} - 10^{-4} (7 experiments) and epinephrine 10^{-3} (3 experiments).

Fig. 18 The comparative effect of *dl*-PMAP and methamphetamine on the decrease of blood flow in a digital artery of dog paw. ●—● *dl*-PMAP (3-7 experiments). ○—○ Methamphetamine (3-8 experiments). The abscissa is a logarithmic scale of a dose in μg . The ordinate is the percentage of the decrease of blood flow. Vertical bar indicates the standard error on one side.

Fig. 17 compares the action of *dl*-PMAP and epinephrine by the whole body perfusion of frogs. At concentrations of *dl*-PMAP between 10^{-7} - 10^{-5} , scarcely any effect was noted while a mild contraction was found at 10^{-4} - 10^{-3} . A definite and persistent vasoconstriction was recognized by the administration of 10^{-2} of *dl*-PMAP. Such an effect was slightly milder than that of methamphetamine at the same concentration. As compared with epinephrine, the effect of *dl*-PMAP was considerably weaker but the duration of action was longer.

With perfusion of a digital artery of dog paw, the administration of 1, 2, 5 and 10 μg of *dl*-PMAP and methamphetamine caused a decrease in blood flow or vasoconstriction. The fall of blood flow in response to *dl*-PMAP was slightly milder than that after methamphetamine on the average. Fig. 18 shows the comparison on the average fall in blood flow in response to *dl*-PMAP and methamphetamine.

In the experiments with perfusion under constant speed, the rise in blood pressure in response to *dl*-PMAP was slightly less than that of methamphetamine.

In the isolated blood vessels of rabbits and dogs, slight vasoconstriction was resulted at a concentration of *dl*-PMAP at 10^{-5} but the intensity of contraction increased as the concentration rose. As compared with the action of methamphetamine at the same concentration, the constricting effect was milder, being less than 1/5 that of epinephrine.

5) Action on the spleen.

In the isolated spleen of rabbits and dogs, concentrations of *dl*-PMAP less than 10^{-3} gave no definite effect but contraction was found above 10^{-2} , as the concentration was increased. Such a contracting effect was somewhat less than that of methamphetamine.

DISCUSSION

Compounds used in this experiment have the side chain identical with that of methamphetamine, with properties closely resembling this. As is evident from the results of the experiments, a change into the piperonyl group may vary the intensity of action and other aspects.

As for the circulatory system, phenyl-dimethylaminopropane produced by N-methylation of the side chain of methamphetamine in the chemical structure had a milder pressor effect and shorter duration of action (Hauschild, 1940, Shimamoto et al., 1955, Matsushima, 1957).

On the other hand, with the substitution of a piperonyl group for the phenyl group, as shown in the present experiment, the pressor effect and its duration lessend in degree and duration compared with methamphetamine but the decrease was less pronounced than with N-methylation.

According to Matsushima (1957), the pressor effect of the *d* isomer of phenyl-dimethylaminopropane was more intense than the *l* isomer. In Mizunoe's

experiment (1961), the pressor effect of the *l* isomer of *dl*-PMAP was weaker than the *d* isomer. The tendency towards a fall in blood pressure was more pronounced in rabbits. According to Goodman et al. (1956), however, the pressor effect of amphetamine was more intense with the *l* isomer than with the *d* isomer, so that it is difficult to assign a constant relationship to the pressor effect of optical isomers of the same compound. Concerning the cardiac action, the action of phenyl-dimethylaminopropane was slightly milder on the isolated heart of frogs, rabbits (Matsushima, 1957) and toads (Shimamoto et al., 1955) with a shorter duration. According to Mizunoe (1961), the substitution of a piperonyl group caused a decrease in action in the isolated heart of frogs and rabbits and the heart of rabbits in vivo. The present experiment also shows the same phenomenon, the *dl* isomer giving an effect intermediate with that of the *d* and *l* isomer alone.

In the experiment on blood pressure of a dog, a transient fall of blood pressure may be seen immediately after the administration of *dl*-PMAP. In the observation on ECG and cardiac action, on the other hand, bradycardia definitely appears. Such a transient fall of blood pressure and bradycardia became milder or disappeared after vagotomy and atropine administration, so that these appear to be based on reflexes through the vagus nerve. The inhibitory effect, however, upon direct application of a large amount to the heart might be partially due to a direct effect on the cardiac muscle.

Concerning the vascular effect of phenyl-dimethylaminopropane in comparison with methamphetamine, reports are available on the decreased contraction of the rabbit ear, the hind limb of toads (Matsushima, 1957), and the vein of cats (Domenjoz et al., 1939). According to Mizunoe's report (1961), scarcely any change in effect was noted between methamphetamine and *dl*-PMAP in the blood vessels of ear of rabbits and hind limb of toads. In the present experiment, however, *dl*-PMAP had a slightly lesser contracting effect on the small vessels in the dog paw.

Against smooth muscles especially intestinal motility in rabbits, phenyl-dimethylaminopropane was reported by various authors to have different effects. According to Hauschild (1940), the inhibitory effect was twice as great as that of methamphetamine. Matsushima (1957) stated that an increase in amplitude and rise of tension was observed immediately after administration.

In the experiment of Mizunoe (1961) and the present experiment, methamphetamine and *dl*-PMAP might produce a transient increase of amplitude at low concentrations but an inhibitory effect was usually seen. The substitution of a piperonyl group slightly weakened the inhibitory effect as compared with methamphetamine.

In experiments on blood pressure in dogs, moreover, the repeated administration of *dl*-PMAP of a constant amount over a constant period was found to produce tachyphylaxis. According to Matsushima (1957), phenyl-dimethylaminopropane in comparison with methamphetamine, has less of a

tendency to cause tachyphylaxis. Mizunoe (1961) was also of the opinion that about twice as much total dose of *dl*-PMAP is required as methamphetamine to cause tachyphylaxis. In the present experiment, the *dl* isomer of PMAP has a lesser tendency to cause tachyphylaxis than methamphetamine. A cross tachyphylaxis was demonstrated between *dl*-PMAP and methamphetamine. With the action on the intestine, on the other hand, Matsushima (1957) and Mizunoe (1961) pointed out the occurrence of tachyphylaxis upon repeated administration of phenyl-dimethylaminopropane or *dl*-PMAP. In the present experiment, similar isolated organs such as the intestine and blood vessels reacted to repeated administration of *dl*-PMAP with decreasing sensitivity. A variety of concepts and experiments are available on the significance and cause of tachyphylaxis and this will be discussed in another paper.

Concerning the mechanism of the pressor effect and actions on various organs of phenylalkylamine, since Gaddum et al. (1938) the theory of inhibition of monoamine oxidase has been supported for a long time. However, Burn et al. (1958) proposed the concept of an indirect action on the receptor through norepinephrine release from the adrenergic nerve terminal. Many studies are being carried out on this point at present. Takasaki (1966 a, c) compared the blood pressure rise in response to reserpine after the administration of indirect acting amines and discussed the mechanism.

In the present experiment, reserpine caused a persistent rise in blood pressure after *dl*-PMAP as with methamphetamine. After reserpine administration, the pressor effect of *dl*-PMAP was markedly intensified and such a rise was depressed or inhibited through the administration of cocaine inhibiting the release of norepinephrine from the adrenergic terminal (Whitby et al., 1960, Hertting et al., 1961, Muscholl, 1961, Weiner et al., 1962, Samorajski et al., 1963, Bhagat, 1963, Hardman et al., 1965) and tolazoline, a blocking agent for adrenergic α receptors. *dl*-PMAP, like methamphetamine, probably exerts its effect through the release of norepinephrine from the adrenergic terminal. However, the releasing action of norepinephrine appears to be weaker in view of the degree of the rise of blood pressure. The nature of the action of *dl*-PMAP on various organs is probably based on the same mechanisms as methamphetamine. On the other hand, a mild blood pressure rise was seen in response to the administration of *dl*-PMAP into the common carotid artery. In the experiments on two dogs by Mizunoe (1961), it is hardly conceivable that these effects are due to a direct central excitation effect. It is more probably due to the release of a small amount of norepinephrine from the adrenergic nerve terminal in the central area. As for the changes in respiration due to *dl*-PMAP, administration into the common carotid artery caused a momentary respiratory excitation. This effect was abolished by denervation of the carotid sinus nerve. This is therefore probably mediated by the sinus nerve. The subsequent inhibition and mild excitation is probably based on a central action.

SUMMARY

Various experiments were conducted on *dl*-piperonyl-methylaminopropane (*dl*-PMAP).

1) The intravenous injection of *dl*-PMAP in dogs caused a rise of blood pressure and a persistent rise was achieved through reserpine administration. However, such a rise was weaker than in methamphetamine. Such pressor effect was inhibited or abolished by cocaine or tolazoline. The repeated administration of *dl*-PMAP caused tachyphylaxis but a larger dose than methamphetamine was required. Moreover, a cross tachyphylaxis was seen between methamphetamine and *dl*-PMAP. In an experiment on blood pressure and ECG changes, bradycardia was definitely observed but disappeared after vagotomy or atropine administration, being mediated reflex by the vagus nerve. However, no special change was found in ECG types.

2) Through intravenous administration of *dl*-PMAP in dogs, respiratory excitation was exhibited, probably through the carotid sinus reflex and a central excitation effect.

3) *dl*-PMAP exerts an facilitating effect on the isolated heart of frogs and rabbits, and rabbit heart in vivo. An inhibitory effect was seen upon the use of large doses.

4) A vasoconstrictive action was observed on the blood vessels of the ear of rabbits, the whole body of frogs and the paw of dogs.

5) In the isolated and intact intestine of rabbits, a slight inhibition of motility was noted. These experimental results indicated a weaker effect of *dl*-PMAP than that of methamphetamine. Repeated administration caused a decreasing of the effect. The action of *dl*-PMAP is quantitatively different from that of methamphetamine in many respects but similar in outline.

The mechanism of action is apparently based on the release of norepinephrine from the peripheral adrenergic nerve, the drug being useful as a mild sympathomimetic amine.

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These experimental results were reported briefly in the 21st meeting of West-South Japanese Pharmacological Society in 1969.

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