EFFECTS OF MONOAMINE OXIDASE INHIBITORS ON THE VASOPRESSOR ACTIONS OF SEVERAL SYMPATHOMIMETIC AGENTS

CHIKAO KONO, ZENGO KANDA, ATSUSHI SEKIYA
AND EIZO SAJI

Department of Pharmacology, Nagoya University School of Medicine

In 1952 Zeller and Bursky showed that iproniazid is a powerful, irreversible inhibitor of monoamine oxidase (M.A.O.) both *in vivo* and *in vitro*.[1] Contrary to their expectations, however, this compound, and other inhibitors as well,[2] failed to potentiate the response to norepinephrine and epinephrine in blood pressure and nictitating membrane. And the so-called "Monoamine oxidase hypothesis"[3] ceased to be acceptable generally. In addition to this, since the discovery of catechol o methyl transferase (C.O.M.T.) by Axelrod *et al,*.[4] it has been admitted that the methylation of metahydroxy group of catecholamines precedes their deaminaton by M.A.O. in the metabolic pathway of sympathomimetic amines.

But afterwards catecholamines were found to be protected by iproniazid and other M.A.O. inhibitors against metabolic inactivation notably in brain[5][6] and in heart,[7] so it may be concluded that M.A.O. probably plays a small but definite part in the metabolism *in vivo* of sympathomimetic amines.

In our laboratory, while studying the physiological and pharmacological properties of M.A.O., one of the authers incidentally discovered that the vasopressor effect of ephedrine and tetrahydrozoline was reduced by pretreating with P.I.H.. So the authors intend to describe in this paper the details of the results of the experiments *in vivo* and *in vitro* which have been undertaken to confirm this phenomenon.

MATERIALS AND METHODS

1. Sympathomimetic drugs and M.A.O. inhibitors

The following sympathomimetic drugs were studied: dl-epinephrine, tetrahydrozoline, l-ephedrine, tyramine and acetylcholine as the hydrochlorides and heptaminol monophosphate. (2-amino-6-hydroxy-6 methyl-heptane)

Two M.A.O. inhibitors were used for the purpose of inhibiting M.A.O. *in vivo*: P.I.H. (1-phenyl-2-hydrazinopropane HCl or J.B. 516, Catron tablets for intraperitoneal injection, the crystalline for intravenous injection.) Phenelzine (phenethylhydazine sulfate, Nardil tablets for intraperitoneal injection, the crystalline for intravenous injection.)

2. Blood pressure measurement

a) Forty-six albino rabbits weighing 2 to 4 kg and 2 young dogs weighing 1.5 kg were used. All rabbits were anesthetised with about 150 mg per kg of phenobarbital and dogs with 25 mg per kg of morphine and 30 mg per kg of phenobarbital subcutaneusly.

Arterial pressure was measured with a mercury manometer from a carotid artery and recorded on smoked paper.

b) Some drugs were administered intravenously with an injection needle inserted and fixed in a femoral vein.

The sympathomimetic drugs were administered in sequence 1 to 3 times during each experiment before the administration of the M.A.O. inhibitor.

The M.A.O. inhibitors were then prepared and administered as follows: The tablets were ground and suspended in distilled water. The suspension containing about 1 mg per ccm of P.I.H. or 3 mg per ccm of phenelzine was injected intraperitoneally. (30 or 50 mg/kg of phenelzine, 2 to 20 mg/kg of P.I.H.) The crystalline form was dissolved in normal saline and administered intraperitoneally (50 mg/kg of phenelzine, 30 mg/kg of P.I.H.) or intravenously so slowly as to minimize unexpected side effect. The doses of the various compounds were expressed as those of their salt.

About 2 hours after P.I.H. was injected intraperitoneally, 3 hours after phenelzine intraperitoneally or an hour after the inhibitors intravenouly, the sympathomimetic drugs were again administered as before.

c) In atropinized dogs the nicotine-like action of acetylcholine was investigated before and after 40 mg per kg of P.I.H. was injected intravenously.

d) The vasopressor response to tetrahydrozoline after administration of reserpine was studied in several rabbits. Reserpine (3 to 5 mg/kg) was administered intraperitoneally 24 hours before the experiment.

3) Monoamine oxidase determinations

a) An hour after P.I.H. was administered intravenously or 2 hours after intraperitoneally, the anesthetised rabbits were sacrificed by intravenous injection of air. The brain and liver were immediately removed, chilled and suspended in 0.067 M phosphate buffer, pH 7.2, by using a Potter-Elvehjem glass homogenizer.

The determination of M.A.O. activity was carried out by the manometric procedure estimating oxygen uptake. The incubation system contained, in a final volume of 2 ml, 0.1 g of liver or 0.33 g of brain as homogenate and 0.01 M tyramine HCl; oxygen was employed in the gas phase. The incubation was carried out at 38° C for 30 minuites.

The percentage of inhibition of M.A.O. activity was calculated as follows:

$$\left(1 - \frac{\text{mean } O_2 \text{ uptake of the liver or brain of M.A.O. inhibited rabbits}}{\text{mean } O_2 \text{ uptake of the liver or brain of intact rabbits (5 cases)}}\right) \times 100\%$$

b) The inhibition of M.A.O. *in vitro* by ephedrine and tetrahydrozoline was studied also by the monometric procedure. The incubation system was prepared as shown in Table 1. The incubation was carried out at 38° C for an

hour. Each value represents a mean oxygen uptake of 2 systems.

TABLE 1. M.A.O. Inhibition *In vitro*

	Tetrahydrozoline		Ephedrine	
	Oxygen uptake	% inhibition of M.A.O.	Oxygen uptake	% inhibition of M.A.O.
M+T	117.8 μl		117.8 μl	
M+I (1/60 mol)	0		0	
M+T+I (1/60 mol)	6.6	94.4%	65.3	44.6%
M+T+I (1/600 mol)	14.7	87.5	103.0	12.6

M: Enzyme suspension, 0.1 g fresh tissue/vessels for liver homogenate.
T : Tyramine 0.067 mol (final concentration.)
I : Inhibitor (Tetrahydrozoline or Ephedrine)
Oxygen was saturated in the gas phase, final volume 3 ml.

RESULTS

1. Blood pressure response to sympathomimetic drugs

The vasopressor actions of the sympathomimetic drugs before and after the administration of M.A.O. inhibitors are shown in Figures 1, 2, 3, 4.   In this experiment no tachyphylaxis of ephedrine and tetrahydrozoline occurred with the used doses.   There was little difference quantitatively in blood pressure response to the sympathomimetic drugs even when the drugs were given at different blood pressure levels.

Ephedrine. After intravenous or intraperitoneal injection of each of the M.A.O. inhibitors the pressor action of ephedrine was significantly reduced both in rabbits and dogs.

Tetrahydrozoline.   The vasopressor response to tetrahydrozoline was reduced after intraperitoneal injection of both inhibitors, but, unlike the case of ephedrine, after intravenous administration of even large doses of P.I.H. it was only moderately reduced.   An explanation of this will be mentioned in the following chapter.

Epinephrine and Heptaminol.   The pressor effects of epinephrine and heptaminol were not significantly affected by the inhibitors.   It has been already reported that heptaminol has pressor effect combining with adrenergic motor receptors and that the compound, however, was unable to compete with epinephrine because of its low affinity for adrenergic receptors.[8]   These observations suggest that the adrenergic motor receptors are still functional even after administration of M.A.O. inhibitors.

Tyramine.   The pressor response to this drug was augmented after administration of each inhibitor.   This potentiation was more apparent in the duration of the effect than in the peak response.

Before P.I.H.   2 hours after P.I.H.

a) Tetrahydrozoline 100 μg/kg

b) Ephedrine 500 μg/kg
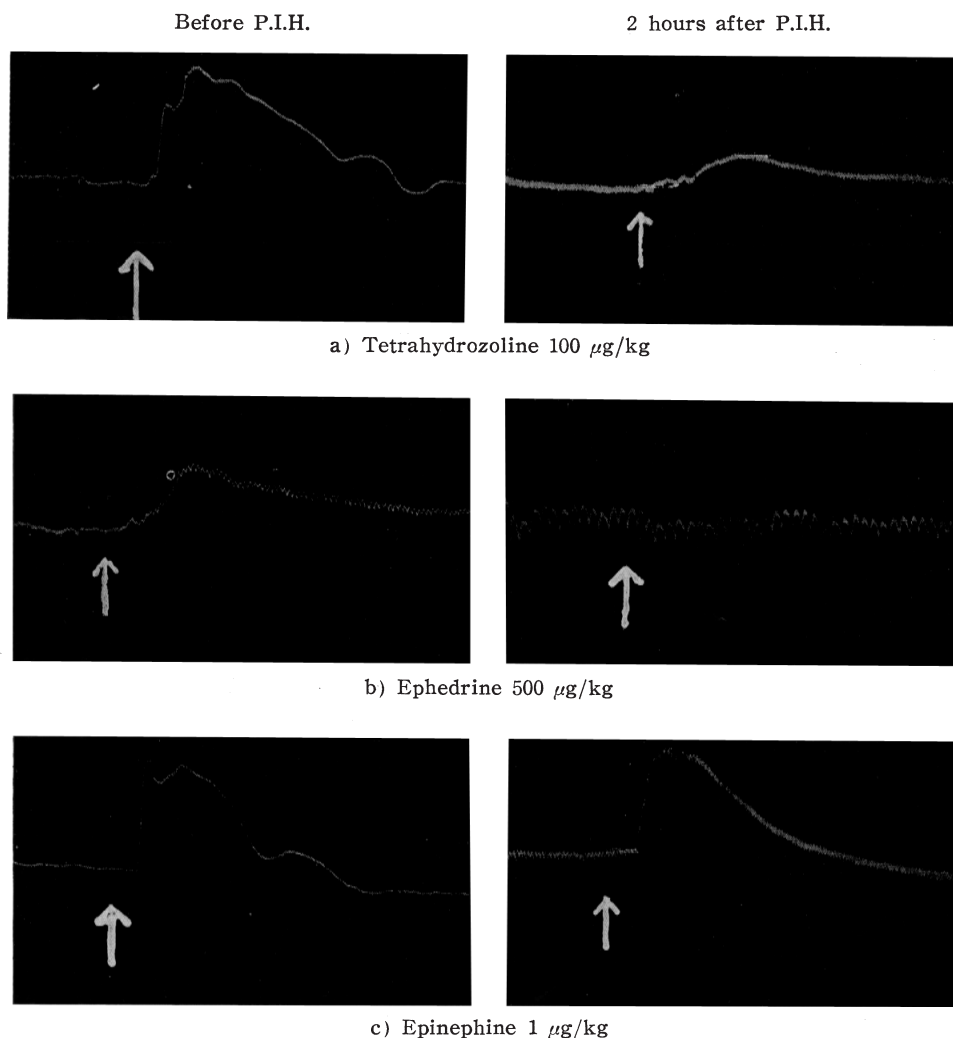

c) Epinephine 1 μg/kg

FIG. 1.   P.I.H., 20 mg/kg, intraperitoneal injection, in rabbits.

Acetylcholine.   In completely atropinized dogs the vasopressor action of acetylcholine was not affected by the inhibitor, so it is supposed that release of epinephrine-like substance at postsynaptic nerve terminals or local chromaffin cells was not impeded.

2) *Miscellaneous actions of the M.A.O. inhibitors*

P.I.H. administered intravenously produced abrupt decrements in heart rate and arterial pressure.   The blood pressure then returned to preinjection levels gradually but the bradycardia frequently persisted for a long time; about 15 to 30 minuites later stimulations of the central nervous system, especially of respiration, were observed.

Before Phenelzine   3 hours after Phenelzine

Ephedrine 500 μg/kg

Heptaminol 20 mg/kg
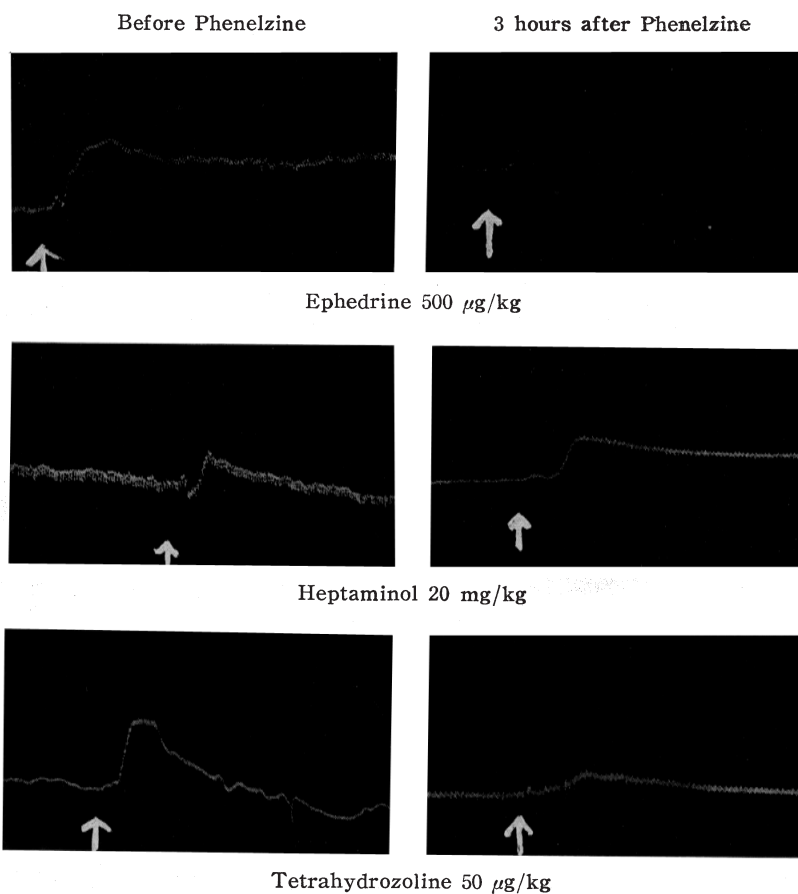

Tetrahydrozoline 50 μg/kg

FIG. 2. Phenelzine 30 mg/kg administered intraperitoneally, in rabbits.

On the contrary, the intraperitoneal administration of P.I.H. had little effect on either heart rate or blood pressure except for a slight stimulation of the central nervous system.

Phenelzine, on the other hand, showed only decrement, though marked, in blood pressure after both intraperitoneal and intravenous adminstration. The decrement in blood pressure persisted for a long time.

3) *Relations between the M.A.O. activity in vivo and the vasopressor response to ephedrine and tetrahydrozoline*

The relative potency of various inhibitors against M.A.O. *in vivo* has recently been demonstrated by Zbinden *et al.*[9] According to their conclusion, the iproniazid quotient of these 2 inhibitors are shown in the following table.

	iproniazid	P.I.H.	phenelzine
liver M.A.O.	1	1	0.14
brain M.A.O.	1	43	12.4

Before P.I.H.   1 hour after P.I.H.

a) Tetrahydrozoline 100 μg/kg

b) Ephedrine 500 μg/kg

c) Tyramine 200 μg/kg

d) Epinephrine 1 μg/kg
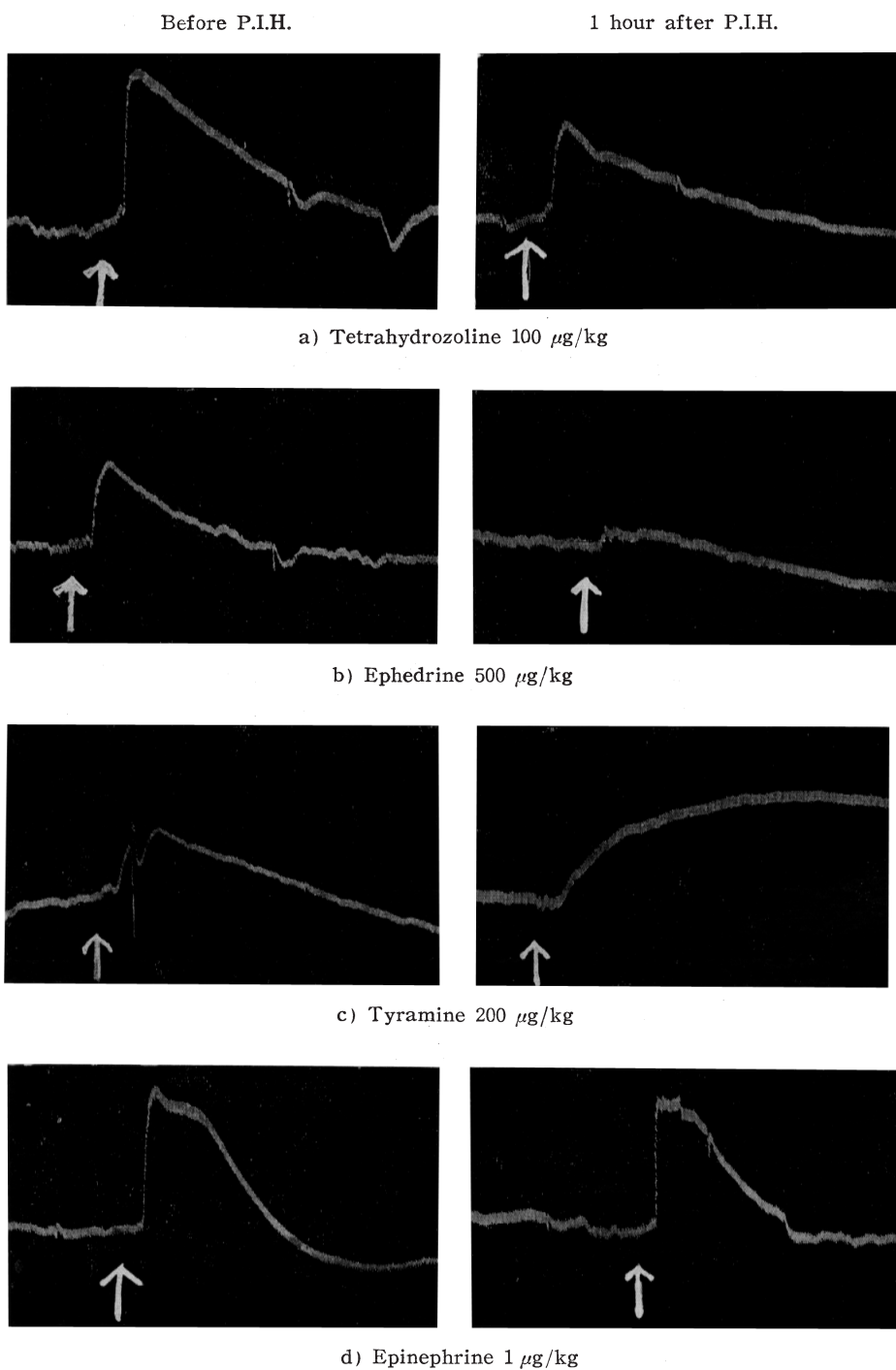
FIG. 3.   P.I.H. 40 mg/kg administered intravenously in rabbits.

Before P.I.H.

1 hour after P.I.H. intravenous injection

0 level

0 level

Ephedrine 500 μg/kg   Tetrahydrozoline 50 μg/kg   Acetylcholine 100 μg/kg

FIG. 4.   P.I.H. 40 mg/kg, intravenous administration, in atropinized dog.
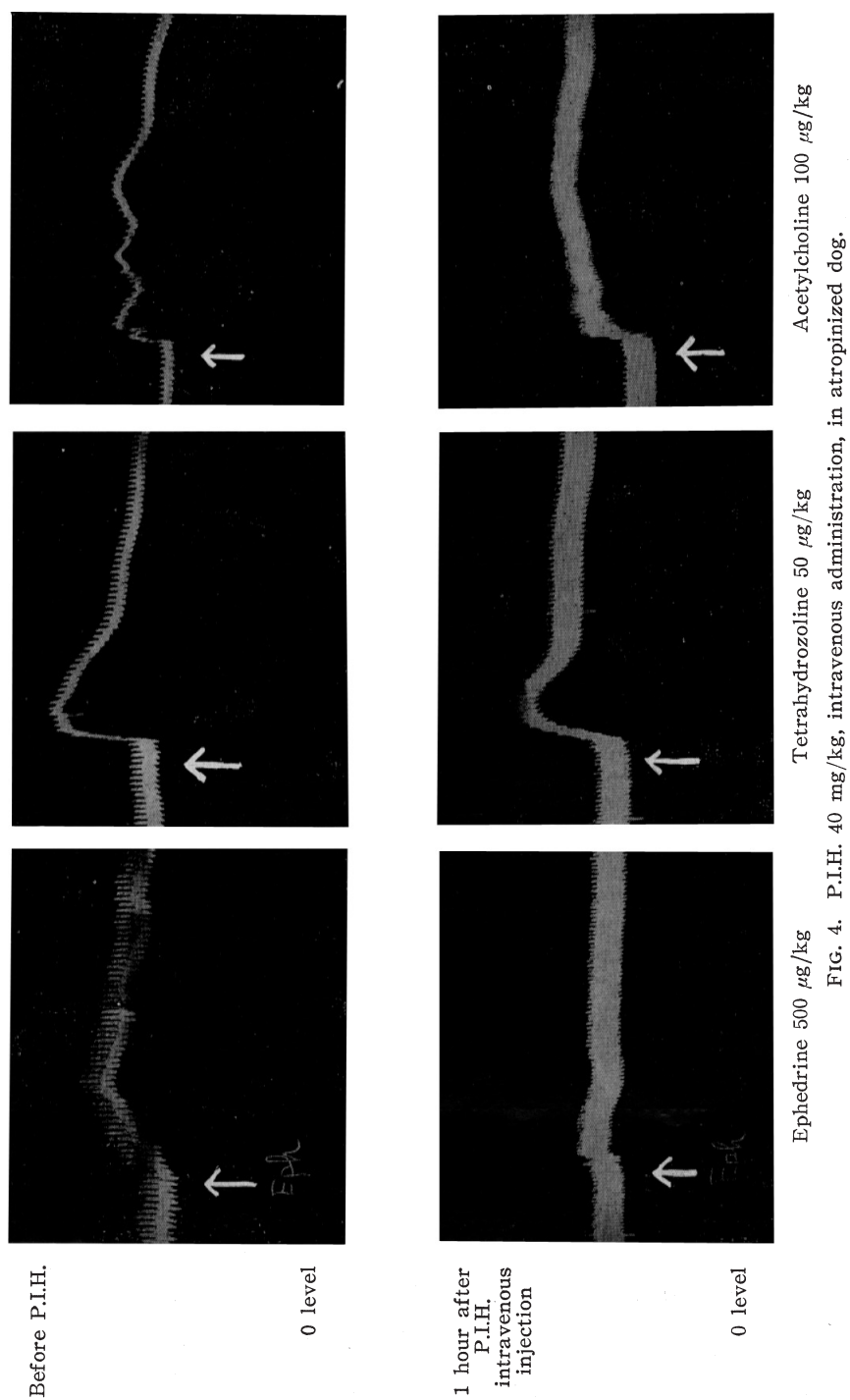
In the present experiments with P.I.H. the inhibition percentage of liver or brain M.A.O. which was compared with normal rabbit M.A.O. activity is shown in Figure 5; and also the reduction of vasopressor response to ephedrine and tetrahydrozoline after P.I.H. was calculated as follows:

Inhibition percentage of blood pressure rising=

$$\left(1-\frac{\text{B.P. increment, mmHg, with sympathomimetics after inhibitors}}{\text{B.P. increment, mmHg, with sympathomimetics before inhibitors}}\right)\times 100\%$$

Each value in Fig. 5 represents a mean of determination on 2 rabbits.
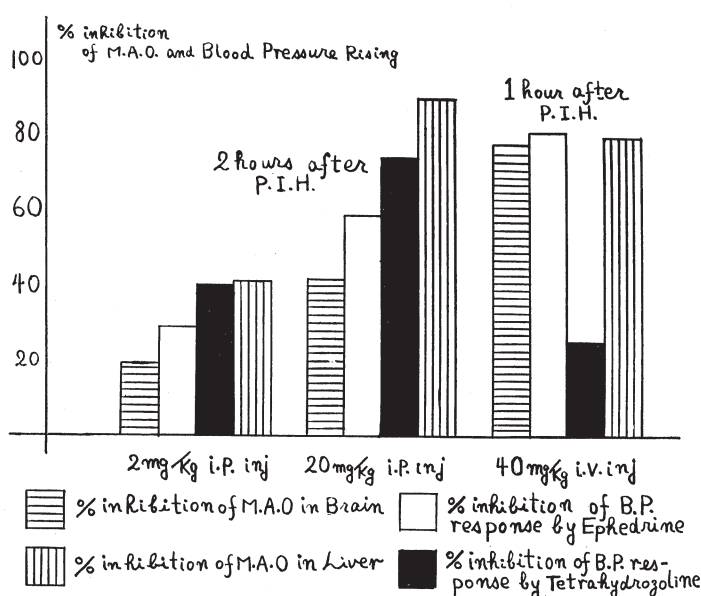B.P.=mean arterial blood pressure.

FIG. 5

Figure 5 illustrates that the degree of inhibition of liver and brain M.A.O. *in vivo* nearly parallels that of the vasopressor response to these sympathomimetic drugs except in the case of the pressor action of tetrahydrozoline after intravenous administration of P.I.H.

4) *M.A.O. inhibition by ephedrine and tetrahydrozoline in vitro*
As shown in Table 1 tetrahydrozoline is the more potent inhibitor of the two.

5) *Vasopressor effect of tetrahydrozoline before and after reserpine*
Unlike ephedrine, reserpinization failed to surpperess the pressor action of tetrahydrozoline.

DISCUSSION

The results of this study demonstrate an interesting pattern of the blood pressure response, following administration of two M.A.O. inhibitors, to six defferent sympathomimetic agents in rabbits and dogs. There was depression or abolition of the pressor actions of ephedrine and tetrahydrozoline, but also prolongation and augmentation of the actions of tyramine. There was no significant change, on the other hand, in the vasopressor response to epinephrine and heptaminol. Also in atoropinized dogs the nicotine-like action of acetylcholine was not affected by administration of the inhibitors in contrast with the results of Gertner's experiment.[10]

The details will be discussed below on each of the drugs examined and summarized in Table 2.

TABLE 2. The Behaviour of Various Sympathomimetic Drugs
Relating with M.A.O.I. and Reserpine

	B.P. Response after M.A.O.I.	B.P. Response after reserpine	Substrate or inhibitor of M.A.O.?
Epinephrine	Not altered	Potentiated	Substrate
Tyramine	Potentiated	Depressed[13]	Substrate
Heptaminol	Not altered[8]	Not altered[8]	No![8]
Ephedrine	Depressed	Depressed[13]	Inhibitor
Tetrahydrozoline	Depressed	Not altered	Inhibitor

Epinephrine: Lack of significant potentiation of vasopressor response to epinephrine by M.A.O. inhibition is consistent with current concepts that efficient routes of metabolism other than by M.A.O. may be available for this catecholamine and, becuase of failure in blockade of the response to epinephrine, it is quite possible that adrenergic receptors are still functional after such doses of two inhibitors.

Heptaminol: As previously reported[8] this substance is neither substrate nor inhibitor of M.A.O. and has only low affinity for adrenergic motor receptors. The failure of M.A.O. inhibitors in blocking the vasopressor effect of this compound suggests that the used doses of the inhibitors have no effect on adrenergic receptors.

Acetylcholine: Recently Gertner reported that ganglion perfusion of P.I.H. blocked transmission through the superior cervical ganglion of cat.[10] Another report demonstrated that iproniazid inhibited release of serotonin from platelets.[11]

From these results, it is supposed that both P.I.H. and phenelzine may inhibit release of catecholamines from sympathetic nerve terminals.

No reduction of the vasopressor response to acetylcholine in atropinized animals, however, suports the view that the release of epinephrine-like substance by acetylcholine is not impeded after intravenous administration of 40 mg per kg of P.I.H..

Ephedrine: Since the early work of Tainter and Burn *et al.*[12] indicated that a large part of sympathomimetic effects of drugs of ephedrine-tyramine group was due to local indirect action, many efforts have been made to reveal the mechanisms of their actions.   In 1938 Gaddum and Kwiathowski introduced that the potentiation of epinephrine responses by ephedrine was due to the inhibitory effect of ephedrine on oxidation epinephrine by M.A.O.

On the other hand, Burn *et al.*[13] reported that the local indirect action of ephedrine is largely attributable to release of norepinephrine-like substance from nerve endings.   Furthermore, in 1953, Axelrod[14] showed that the systemic actions of ephedrine were largely through norephedrine which was a metabolite of ephedrine. More recently Sano[15] demonstrated that phenylmethylaminopropane was a very effective inhibitor of epinephrine transport into blood platelets.

At the present time, however, there is no acceptable explanation for the mechanism of actions of compounds belonging to the ephedrine-tyramine group. The possibility exists that the sympathomimetic effect of ephedrine may be due to combination of more than one type of the mechanisms indicated above and some others.   Unlike the case of physostigmine, it is generally accepted that ephedrine does not exert its pharmacological activity by inhibiting M.A.O.

In our experiment, however, the pressor effect of ephedrine was reduced by the pretreatment with M.A.O. inhibitors, P.I.H. or Phenelzine, without any inhibitory effect of P.I.H. on the release of epinephrine and any competition of inhibitors with epinephrine for adrenergic receptors.   Furthermore, there was seen a rough parallelism between the degree of decrement in the pressor effect of ephedrine and that of inhibition of the M.A.O. activity *in vivo*.   So it is assumed that the vasopressor effect of ephedrine is in part due to the interaction between the M.A.O. *in vivo* and ephedrine.

The inhibitory effect of M.A.O. inhibitors on the vasopressor action of ephedrine is similar to that of cocaine which was reported by Fleckenstein *et al.*[16]   In 1958 Burn and Rand suggested that the action of cocaine was probably to arrest the release of the noradrenaline-like substance from the store site.[13] But more recently Trendelenburg[17] demonstrated that cocaine neither increased nor decreased output of splenic sympathin in cat.

From the facts reported by Trendelenburg and illustrated in Figure 4 it is conceivable that the inhibitory effect of cocaine or M.A.O. inhibitors on the vasopressor response to ephedrine may not be due to their arrest of epinephrine release induced by ephedrine from nerve terminals with the used doses.

However, on the question whether the mechanism of these two phenomena are the same or not and on the "monoamine oxidase hypothesis", further studies must be carried out.

Tetrahydrozoline: Between ephedrine and this drug there are pronounced differences in the effect on the cardiovascular system.   Tetrahydrozoline produces a slowing of the heart rate, decrease in cardiac minute-volume and increase in peripheral vascular resistance.[18]   Shinagawa[19] showed that this compound exerts its pharmacological effects by both direct and indirect action— the former is initiated by its direct combination with adrenergic receptors and the latter produced by other mechanisms.

Besides these facts, it was reported that tetrahydrozoline itself was an adrenergic blocking agent.[20]   In the present experiment the vasopressor response to tetrahydrozoline was not affected by reserpine.

Nevertheless, the mode of action of tetrahydrozoline is quite similar to that of ephedrine in the point that the vasopressor effect of tetrahydrozoline was reduced by administration of the M.A.O. inhibitors and there was also a similar parallelism to that in the case of ephedrine especially when the crystalline solution or the ground tablet suspension of the inhibitors was administered intraperitoneally. As noted before, however, the intravenous administration of P.I.H. yielded only a slight depression of the action of tetrahydrozoline.   To account for this fact an explanation will be offered: A) Tetrahydrozoline has both direct and indirect actions on adrenergic motor receptors; B) but has no cardiotonic effect and its pressor action is caused by its vasoconstrictor effect. C) Furthermore it is assumed that the concentration of inhibitors at the vascular smooth muscle of abdominal organs which are related with blood pressure level in the body may be selectively maintained at higher level by intraperitoneal administration than by intravenous injection of the inhibitors, and thus the unsurmountable inhibition of M.A.O. activity may occure at the vascular beds. On the contrary, when the inhibitors were injected intravenously, it is possible that the concentration of the inhibitors at abdominal vascular beds may not be sufficiently high to produce unsurmountable inhibition of M.A.O. activity even with a large dose of inhibitors and thus tetrahydrozoline can react to M.A.O. because of its high affinity for M.A.O.. These may be reasons why the inhibitors administered intravenously fails in the blockade of the vasopressor effect of tetrahydrozoline.

Tyramine: The augmentation of the actions of tyramine by M.A.O. inhibitors suggests that the mechanism of actions of tyramine may be different from that of ephedrine.

It will be wise, however, to keep in mind the possibility that, as Burn *et al.* have noted,[13] there may also be similarity between the mechanism of action of tyramine and that of ephedrine.

<div align="center">SUMMARY AND CONCLUSION</div>

The effect of two monoamine oxidase inhibitors (P.I.H. and Phenelzine) on the vasopressor response to several sympathomimetic drugs were studied in the anesthetised animal and the mechanisms of actions of these sympathomimetic drugs were discussed.

1. The actions of ephedrine and tetrahydrozoline on arterial pressure were significantly reduced by both inhibitors. It is posturated that this reduction may be closely related to M.A.O. inhibition.

2. The actions of epinephrine and heptaminol were not significantly altered by the administration of the inhibitors.

3. The nicotine-like action of acetylcholine was not blocked by P.I.H. This suggests that the release of catecholamine may not be reduced by small doses of P.I.H..

4. The vasopressor effect of tyramine were augmented by the inhibitors.

5. Other actions of both inhibitors differed considerably and some of them were probably unrelated to the M.A.O.-blocking activity of these two compounds.

REFERENCE

1. ZELLER. E. A. AND J. BARSKY: *Proc. soc. exp. biol. N. Y.* **81**: 459, 1951.
2. GORDBERG, L. I. AND A. SJOERDSMA: *J. Pharmacol. exper. therap.* **127**: 212, 1959.
3. GADDUM, J. H. AND H. KWIATHOWSKI: *J. Physiol.* **94**: 87, 1938.
4. AXELROD, J: *Science* **126**: 400, 1957.
5. SANO, I. AND K. TANIGUCHI: *Nihon Rinsho* **17**: 1801, 1959 (Japanese).
6. SHORE, P. A., J. A. MEAD, R. G. KURTZMAN, S. SPECTOR AND B. B. BRODIE: *Science* **126**: 1063, 1957.
7. PLETSCHER, A.: *Experimentia.* **114**: 17, 1958.
8. KONO, C.: *Folia. Pharmacol. Japon* **57**: 399, 1961 (Japanese).
9. ZBINDEN, G., L. O. RANDALL AND R. A. MOE: *Dis. Nerv. Syst.* **21**: 89, 1960 (cited from The Saishin-Igaku), (Japanese).
10. GERTNER, S. B. *Nature Lond.* **183**: 750, 1959.
11. PAASONEN, M. K. AND A. PLETSCHER: *Experimentia.* **16**: 30, 1960.
12. TAINTER, M. L. AND J. H. BURN: *Cited from Pharmacol. Rev.* **7**: 246, 1955.
13. BURN, J. H. AND M. J. RAND: *J. Physiol.* **144**: 314, 1958.
14. AXELROD, J.: *J. Pharmacol. exper. therap.* **109**: 62, 1953.
15. SANO, I., Y. KAKIMOTO, K. TANIGUCHI AND M. TAKEDA: *Am. J. Physiol.* **197**: 81, 1959.
16. FLECKENSTEIN, A. AND D. STÖCKLE: *Arch. exp. Path. Pharmakol.* **224**: 55, 1955.
17. TRENDELENBURG, U.: *J. Pharmacol. exper. therap.* **125**: 55, 1959.
18. HUTCHEON, D. E., A. SCRIABINE AND V. N. NIESLER: *J. Pharmacol. exper. therap.* **122**: 101, 1958.
19. SHINAGAWA, H.: *Folia. Pharmacol. Japon* **53**: 828, 1957 (Japanese).
20. HUTCHEON, D. E., S. Y. PLAN AND J. F. GRADOCKI: *J. Pharmacol. exper. therap.* **113**: 341, 1955.