Br. J. Pharmac. Chemother. (1967), **30**, 67–77.

AUTO-POTENTIATION AND POTENTIATION BY INHIBITION OF MONOAMINE OXIDASE OF THE SYMPATHOMIMETIC ACTION OF PHENELZINE AND PHENIPRAZINE

BY

VAN CLINESCHMIDT AND AKIRA HORITA

From the Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington

(*Received November* 4, 1966)

Phenelzine (β-phenylethylhydrazine) and pheniprazine (α-methyl-β-phenylethylhydrazine) are primarily recognized as inhibitors of the enzyme monoamine oxidase, although both drugs also exert an indirect sympathomimetic effect through the release of endogenous catecholamines (Eltherington & Horita, 1960 ; Lee, Shin & Shideman, 1961 ; Tsai & Fleming, 1965).

The present investigation was prompted by the observation of Chessin, Dubnick, Leeson & Scott (1959) of a progressive increase in the vasopressor activity of repeated injections of 0.6 mg/kg phenelzine into the anaesthetized dog and by the finding (Horita, 1965) that the initial inactivation of phenelzine, both *in vitro* and in the intact animal, occurs through a monoamine oxidase-catalysed reaction. As shown by Horita (1965), an initial dose of phenelzine will slow the inactivation of a subsequent dose. In contrast to phenelzine, pheniprazine is not inactivated by monoamine oxidase (Horita, 1963). Therefore, in order to examine the possibility that phenelzine exhibits auto-potentiation because of its ability to function both as a substrate for and as an inhibitor of monoamine oxidase, responses of the blood pressure and nictitating membrane to injections of phenelzine and pheniprazine are compared in control and nialamide pretreated cats.

METHODS

Cats of either sex weighing 2 to 3 kg were divided into control and pretreated groups. Animals in the pretreated group received an intraperitoneal injection of 22.4 mg/kg nialamide HCl, an inhibitor of monoamine oxidase, 18 to 22 hr before the experiments. Nialamide was the monoamine oxidase inhibitor of choice for pretreatment because it lacks the sympathomimetic property inherent in many other inhibitors of monoamine oxidase (Ryall, 1961).

Pentobarbital (30 mg/kg intraperitoneally) anaesthesia, tracheal cannulation, placement on a mechanical respirator (75 ml., 15/min), right femoral vein cannulation for drug administration, catheterization of the left common carotid artery for arterial pressure measurement, and bilateral vagotomy were performed in that order. The head of the animal was secured to the operating table, and the nictitating membrane was fastened to a light thread *via* a small heart clip (Palmer, C145). The thread was passed under a free running pulley to a Grass Force-Displacement Transducer (Model

FT 03). The eye was not enucleated. Contractions of the membrane were determined after placing it under an initial tension of 5 *g*. Instrumentation consisted of either a Gilson Polygraph (Model M5P), Statham Pressure Transducer (Model P23AA), and Grass Force-Displacement Transducer or a Physiograph " Four " recorder, E & M Pressure Transducer (Model P-1000), and E & M Myograph (Type B). Mean arterial blood pressure was recorded directly on the Gilson apparatus and calculated from the Physiograph recordings of pulse pressure according to the formula: mean pressure = diastolic pressure + 1/3 pulse pressure.

Phenelzine sulphate (4 mg/kg) or pheniprazine HCl (0.5 mg/kg) in a volume of 1 ml. distilled water, was administered into the right femoral vein at 15 min intervals and always followed by 1 ml. 0.9% (w/v) sodium chloride solution. The above doses of phenelzine and pheniprazine were selected because preliminary observations indicated that they produced near equal initial contractions of the nictitating membrane and near equal pressor responses in control cats.

The significance or non-significance of the difference between the mean values of the various measurements was determined using Student's *t* test. A P value of <0.05 was considered significant.

Another group of cats was used to determine the extent of monoamine oxidase inhibition existing at 22 hr after pretreatment with nialamide. The heart and portions of the liver from control and pretreated cats were rapidly removed, blotted, and weighed. The tissues were ground separately with sufficient chilled 0.9% saline to make a final concentration of 33% (w/w). One ml. of the homogenate was added to a flask containing 0.3 ml. 0.067M phosphate buffer (pH 7.4) and 0.7 ml. distilled water. This mixture was incubated for 15 min at 37° C, after which 1 ml. 5-hydroxytryptamine creatinine sulphate solution (6 μ-mole/ml.) was added to the flask. The incubation was continued for an additional 40 min. The residual 5-hydroxytryptamine was extracted and assayed according to the colorimetric method of Udenfriend, Weissbach & Brodie (1958).

RESULTS

Extent of monoamine oxidase inhibition resulting from nialamide pretreatment

In order to establish the effectiveness of pretreatment with nialamide, the activity of monoamine oxidase of homogenates from control and pretreated animals was investigated. Duplicate determinations of monoamine oxidase activity were performed on each homogenate of liver and heart from three control cats and three cats administered nialamide base (20 mg/kg) 22 hr previously. In the control liver there was a mean metabolism of 5-hydroxytryptamine for the six determinations of 53.1% (S.E. = $\pm$2.4), whereas homogenate of the liver from nialamide treated animals was able to metabolize only 2.4% (S.E. = $\pm$1.9) of the 5-hydroxytryptamine available in the incubation flask.

The sensitivity of the method suggests that liver monoamine oxidase was about 100% inhibited in cats pretreated with nialamide. Monoamine oxidase activity—that is, ability to metabolize 5-hydroxytryptamine—could not be demonstrated with homogenate of heart from any of the three control or three nialamide pretreated cats, supporting Bernheim & Bernheim (1945) who found little monoamine oxidase in cat heart.

Nictitating membrane contractions to injections of phenelzine into control and nialamide pretreated cats

Figure 1 shows the responses of a typical control cat to intravenous injection of several doses of phenelzine sulphate (4 mg/kg) at 15 min intervals. Not only the magnitude but also the duration of the contraction became progressively greater. The second response was larger than the first, the third was larger than the second, and the fourth, fifth, and sixth responses were larger than their immediately preceding responses, even though the

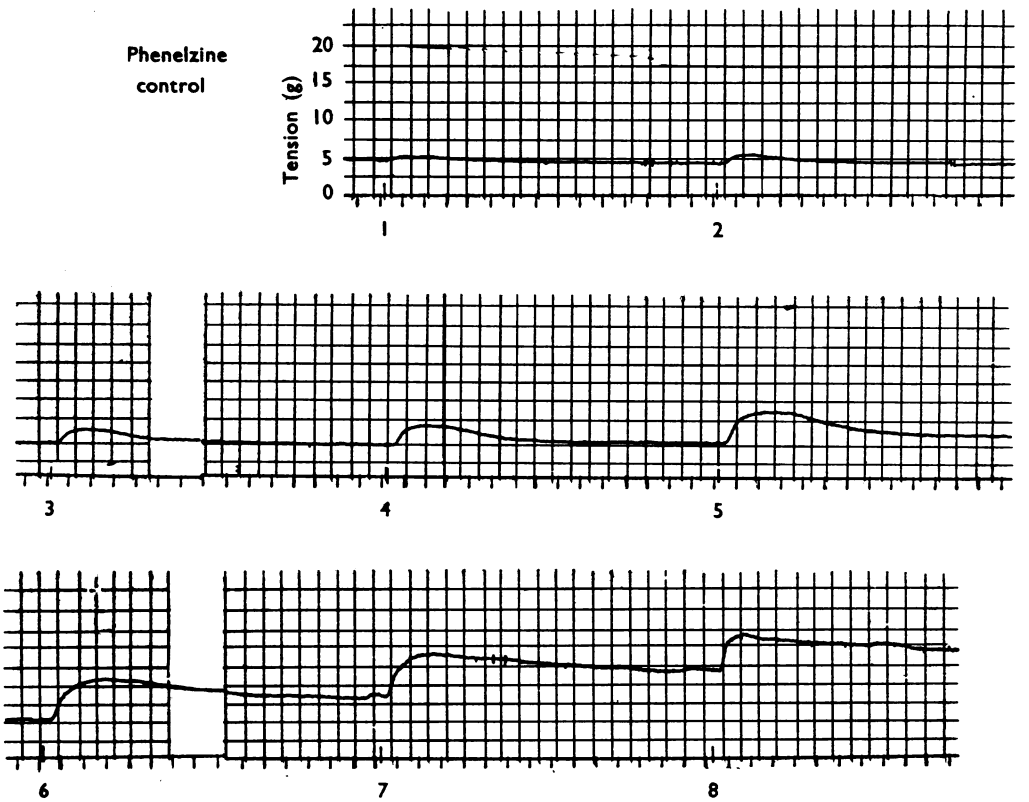

Fig. 1. A record of the tension of the nictitating membrane of a control cat receiving phenelzine sulphate (4 mg/kg intravenously) at intervals of 15 min. Numbers 1 to 8 refer to the succession of the individual doses. Tension is scaled vertically in g and time proceeds horizontally in min.

baseline before each injection had returned to the 5 g resting level. Phenelzine, therefore, is self-potentiating in that prior doses enhance the response to subsequent doses.

In a cat pretreated with nialamide (Fig. 2), the contractile response to the first injection of 4 mg/kg phenelzine sulphate was much larger and of longer duration than the first response in the control animal. The subsequent injections of phenelzine produced only small changes in tension of the nictitating membrane, possibly because these responses were superimposed on an elevated baseline.

The results obtained from five control and five nialamide pretreated animals are summarized graphically in Fig. 3. The tension of the nictitating membrane increased in control cats with each succeeding dose, whereas in those animals pretreated with nialamide the first injection of phenelzine produced a large rise in tension of the membrane and later doses were capable of only approximately reaching or maintaining the initial level of contraction.

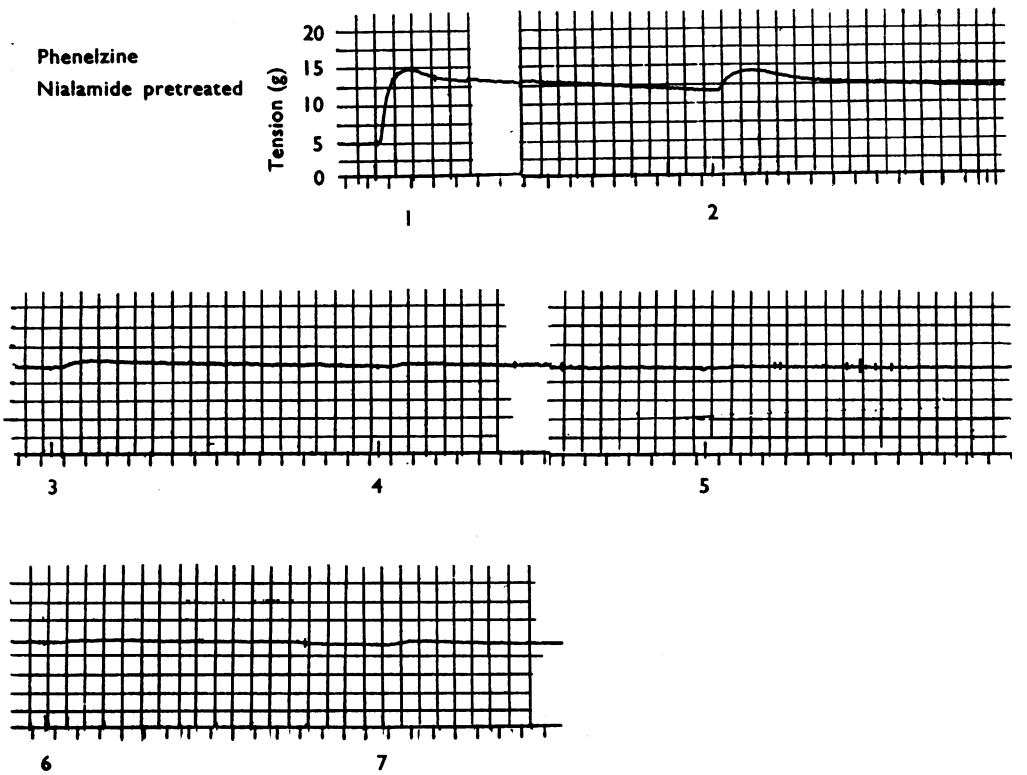

Fig. 2. A record of the tension of the nictitating membrane of a nialamide pretreated cat (20 mg/kg 19 hr previously) receiving phenelzine sulphate (4 mg/kg) at 15 min intervals. Numbers 1 to 7 indicate the succession of the individual injections. Tension is in g and the time marks are 1 min.

Nictitating membrane contractions to injections of pheniprazine into control and nialamide pretreated cats

Intravenous injections of several doses of pheniprazine HCl (0.5 mg/kg) at 15 min intervals resulted in contractile response patterns similar to those produced with administration of phenelzine. The second, third, and fourth responses were larger than their immediately preceding responses, even though the baseline before each injection was 5 g (Fig. 4). Thus pheniprazine demonstrates auto-potentiation.

In a cat pretreated with nialamide (Fig. 5), the first injection of pheniprazine HCl (0.5 mg/kg) caused a large rise in membrane tension. Subsequent doses of pheniprazine were able to elevate the tension of the membrane to levels only slightly larger than the greatest tension produced during the first injection.

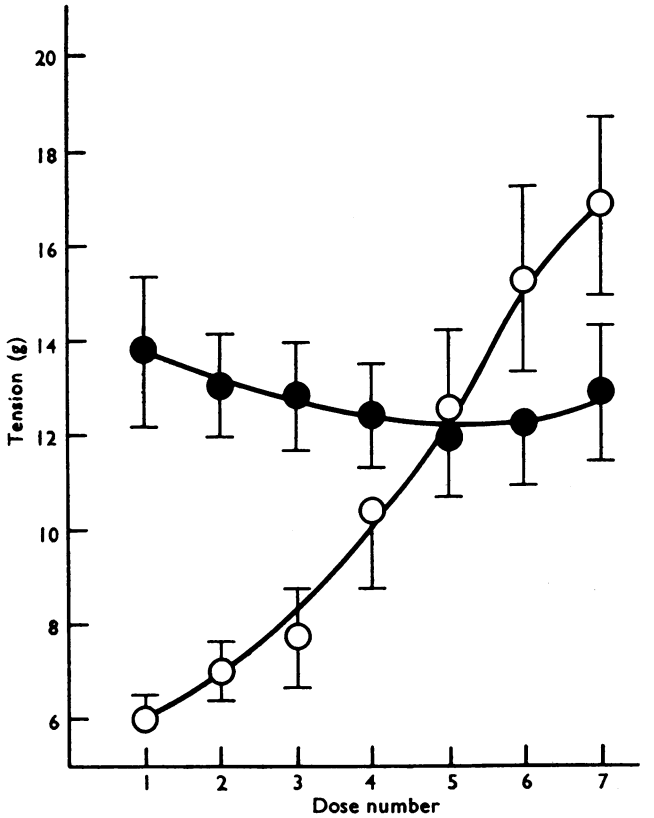

Fig. 3. Average tension of the nictitating membrane in control (○——○) and nialamide pretreated (●——●) cats (20 mg/kg 18–22 hr previously) receiving phenelzine sulphate (4 mg/kg) at 15 min intervals. Each point shows the greatest tension, regardless of its duration or the pre-existing baseline, attained after each injection, and each point is the mean value from five control or five pretreated cats. Vertical bars are ±S.E. Dose number refers to the succession of the individual injections. There is a significant difference between doses 1 ($P<0.01$), 2 ($P<0.01$) and 3 ($P<0.02$) of the control and the pretreated groups.

The results obtained from eight control and five nialamide pretreated cats are summarized graphically in Fig. 6. The tension of the nictitating membrane increased with each succeeding injection of pheniprazine into control cats, whereas in those animals pretreated with nialamide the first injection of pheniprazine caused a large rise in tension of the membrane and later doses were capable of only approximately reaching or maintaining the initial level of contraction.

Pressor response to phenelzine and pheniprazine in control and nialamide pretreated cats

Neither phenelzine (4.0 mg/kg) nor pheniprazine (0.5 mg/kg) exhibited auto-potentiation of their pressor responses in control animals.

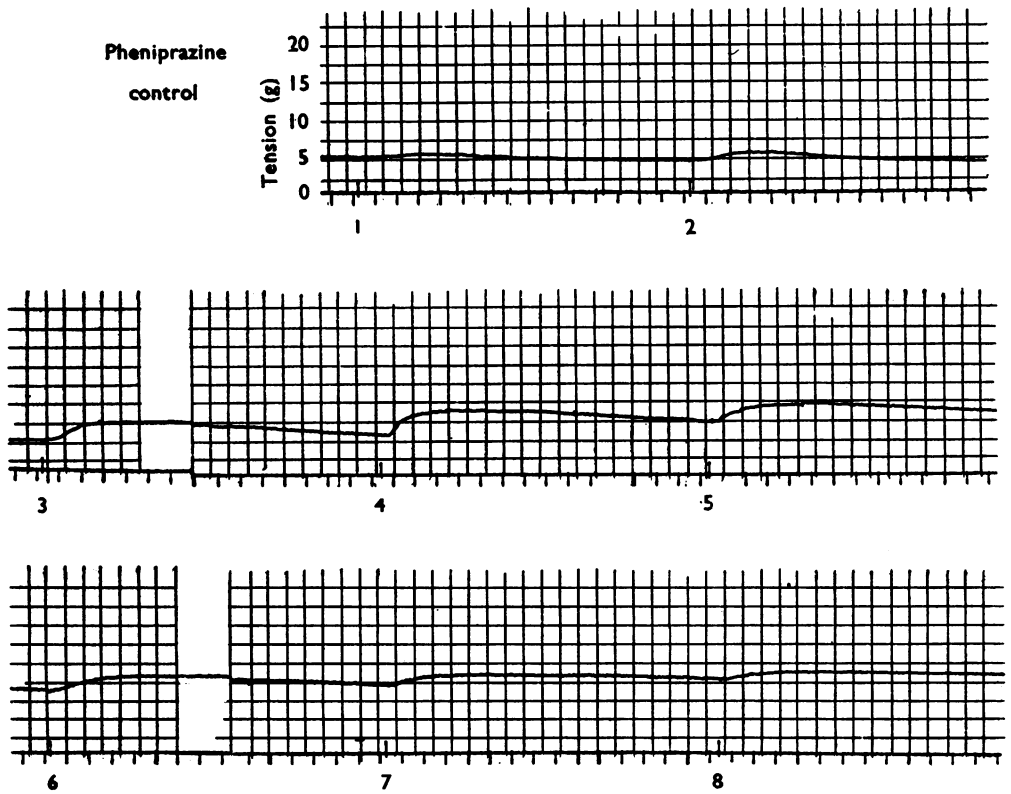

Fig. 4. A record of the tension of the nictitating membrane of a control cat receiving pheniprazine HCl (0.5 mg/kg) at intervals of 15 min. Numbers 1 to 8 refer to the order of the individual doses. Tension is in g and time is marked in min.

Pretreatment with nialamide hastened the development of tachyphylaxis to the pressor action of phenelzine (Fig. 7), but had no significant effect on the pressor action of pheniprazine. Day & Rand (1963) observed that cats pretreated with nialamide became tachyphylactic to injections of tyramine or phenylethylamine (both substrates of monoamine oxidase) at a more rapid rate than control animals.

DISCUSSION

Phenelzine is inactivated by a system insensitive to cyanide, located in the mitochondrial fraction of the cell, dependent upon oxygen, abolished by boiling, and inhibited by various inhibitors of monoamine oxidase (Horita, 1965). From these and other findings it was concluded that monoamine oxidase or a similar system was involved in the inactivation of phenelzine. The results reported here reveal that both phenelzine and pheniprazine potentiate the sympathomimetic action of themselves on the cat nictitating

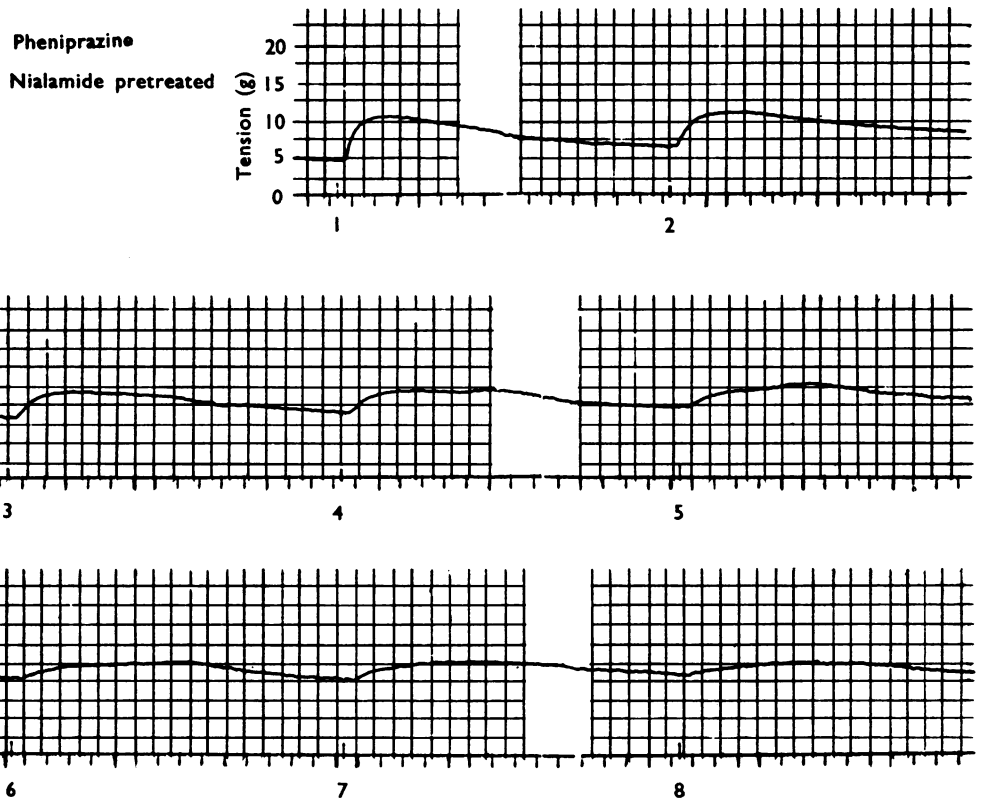

Fig. 5. A record of the tension of the nictitating membrane of a nialamide pretreated cat (20 mg/kg 21.5 hr previously) receiving pheniprazine HCl (0.5 mg/kg) at intervals of 15 min. Numbers 1 to 8 indicate the order of the individual injections. Tension is scaled in g and time is marked in min.

membrane. If pheniprazine could also be inactivated by the monoamine oxidase catalysed route, then auto-potentiation by both pheniprazine and phenelzine would be explicable as inhibition by each drug of its own metabolism. Prior results from our laboratory indicate that monoamine oxidase does not participate in the inactivation of pheniprazine. Boiling of rat liver homogenate before the addition of either phenelzine or pheniprazine caused an increase in the ability of the homogenate to inactivate pheniprazine, whereas the ability to inactivate phenelzine was lost (Horita, 1963, 1965). The increase in the inactivation of pheniprazine by boiled tissue, plus the failure of pheniprazine to fulfil the structural requirement of a substrate for monoamine oxidase of an unsubstituted ethylene side chain (Blaschko, 1952), makes it unlikely that monoamine oxidase itself could serve as a common system for inactivation of phenelzine and pheniprazine.

Phenelzine and pheniprazine are inhibitors of monoamine oxidase, and inhibition of monoamide oxidase by pretreatment of cats with nialamide was found to potentiate the contraction of the nictitating membrane to the first injection of phenelzine or

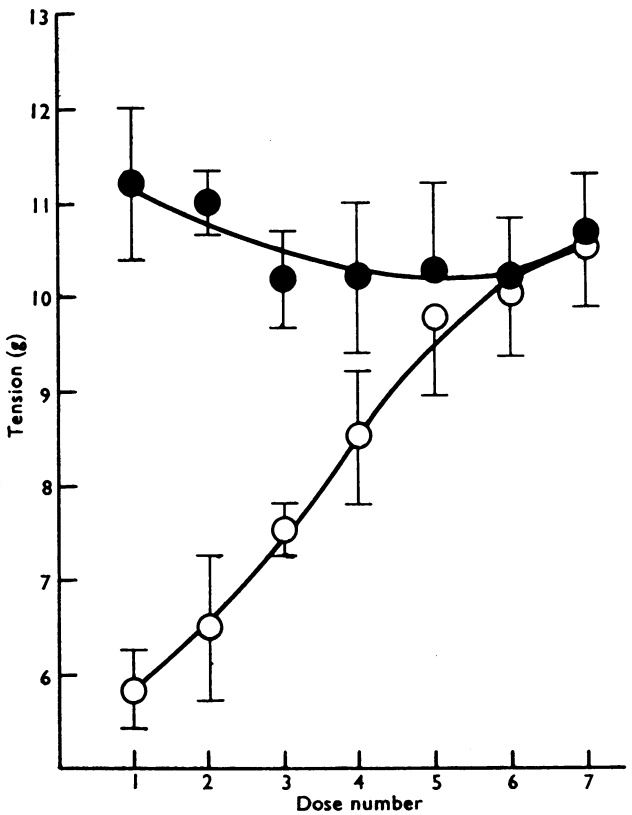

Fig. 6. Average tension of the nictitating membrane in control (○——○) and nialamide pretreated (●——●) cats (20 mg/kg 18–22 hr previously) receiving pheniprazine HCl (0.5 mg/kg) at 15 min intervals. Each point shows the greatest tension, regardless of its duration or the pre-existing baseline, attained after each injection, and each point is the mean value from eight control or five pretreated cats. Vertical bars are ±S.E. Dose number refers to the succession of the individual injections. There is a significant difference between doses 1 ($P<0.001$), 2 ($P<0.001$) and 3 ($P<0.01$) of the control and the pretreated groups.

pheniprazine. Therefore, in control animals, inhibition of monoamine oxidase occurring as a consequence of the initial doses of phenelzine or pheniprazine might be responsible for the enhancement of the effects of later injections of the drugs. In view of recent reports (Holtz & Palm, 1965 ; Smith, 1966) indicating that a portion of the noradrenaline released by indirect sympathomimetic amines is deaminated intraneuronally by monoamine oxidase, it appears within reason to speculate that phenelzine and pheniprazine auto-potentiation is, at least in part, the consequence of gradual inhibition of monoamine oxidase and thereby gradual attenuation of intraneuronal deamination of the noradrenaline they release. It is unlikely that pretreatment of the animals with nialamide caused an enhancement of the response of the nictitating membrane to the first injection of pheniprazine or phenelzine by allowing more catecholamine to become available for

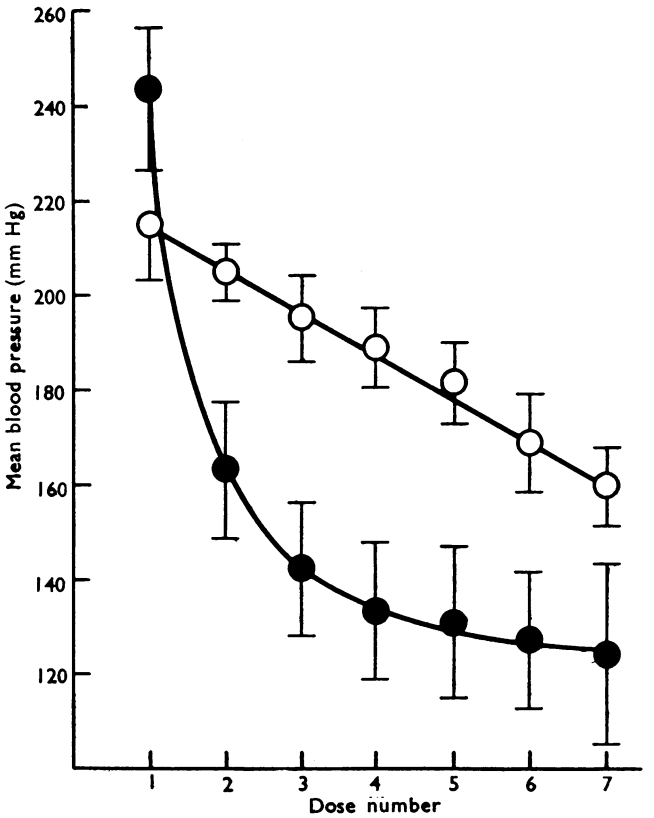

Fig. 7. Pressor responses to phenelzine (4 mg/kg) at 15 min intervals, in control (○——○) and nialamide pretreated (●——●) cats (20 mg/kg 18–22 hr previously). Each point shows the greatest blood pressure, regardless of duration, reached after each injection. In both groups, the time interval of 15 min between injections was sufficient for the baseline to return to its original value prior to each dose of phenelzine. Each point is the mean value from five control or five pretreated animals, and the vertical bars are ±S.E. Dose number refers to the order of the individual injections. There is a significant difference between doses 2 ($P<0.05$), 3 ($P<0.02$), 4 ($P<0.01$), 5 ($P<0.05$), and 6 ($P<0.05$) of the control and the pretreated groups.

release, inasmuch as inhibitors of monoamine oxidase actually lower or do not significantly alter the catecholamine content of cat heart (Euler & Hellner-Björkman, 1955; Goldberg & Shideman, 1962; Davey, Farmer & Reinert, 1963), spleen (Euler & & Hellner-Björkman, 1955; Davey *et al.*, 1963), and nictitating membrane (Cervoni, P., personal communication).

Unfortunately, the present results do not clearly indicate if phenelzine and pheniprazine potentiate themselves by the same mechanism. A common mechanism seems possible, inasmuch as prior inhibition of monoamine oxidase with nialamide enhanced the initial effect of both drugs on the nictitating membrane. On the other hand, nialamide pretreatment accelerated the development of tachyphylaxis only to the pressor action

of phenelzine, and since indirect acting sympathomimetic amines which are well known as substrates of monoamine oxidase are similarly affected (Day & Rand, 1963), the inactivation of phenelzine by monoamine oxidase seems to proceed on a physiological level. Therefore, auto-inhibition of inactivation appears to provide some part of the explanation of the mechanism whereby phenelzine potentiates the sympathomimetic action of itself.

SUMMARY

1. Responses of the blood pressure and nictitating membrane to repeated injections of phenelzine and pheniprazine were compared in control and nialamide pretreated cats.

2. Tachyphylaxis to the pressor action of phenelzine developed more rapidly in animals pretreated with nialamide, whereas the pressor action of pheniprazine was not affected by inhibition of monoamine oxidase.

3. Successive injections of phenelzine or pheniprazine into the control cat resulted in contractions of the nictitating membrane that gradually increased in amplitude and became more prolonged in duration.

4. Following inhibition of monoamine oxidase with nialamide, the first injection of either phenelzine or pheniprazine produced a large contraction of the nictitating membrane, and further injections were not capable of increasing the membrane tension beyond the level attained with the first dose.

5. The results of the blood pressure experiments are consistent with phenelzine, but not pheniprazine, serving as a substrate for monoamine oxidase.

6. The experiments on the nictitating membrane indicate that phenelzine and pheniprazine potentiate their own sympathomimetic action because of their ability to inhibit monoamine oxidase. In the case of phenelzine, auto-potentiation may be, at least in part, a reflection of the inhibition by phenelzine of its inactivation by monoamine oxidase.

The authors wish to acknowledge Pfizer & Co., Inc., Groton, Connecticut, for supplies of nialamide (Niamid®), Lakeside Laboratories, Inc., Milwaukee, Wisconsin, for pheniprazine (Catron®), and the Warner-Lambert Research Institute, Morris Plains, New Jersey, for phenelzine (Nardil®). This study was supported in part by grants No. 5 T1 GM 109-07 and MH 02435 from the United States Public Health Service.

REFERENCES

BERNHEIM, F. & BERNHEIM, M. L. C. (1945). The inactivation of tyramine by heart muscle *in vitro*. *J., biol. Chem.*, **158**, 425–431.

BLASCHKO, H. (1952). Amine oxidase and amine metabolism. *Pharmac. Rev.*, **4**, 415–458.

CHESSIN, M., DUBNICK, B., LEESON, G. & SCOTT, C. C. (1959). Biochemical and pharmacological studies of β-phenylethylhydrazine and selected related compounds. *Ann. N.Y. Acad. Sci.*, **80**, 597–608.

DAVEY, M. J., FARMER, J. B. & REINERT, H. (1963). The effects of nialamide on adrenergic functions. *Br. J. Pharmac. Chemother.*, **20**, 121–134.

DAY, M. D. & RAND, M. J. (1963). Tachyphylaxis to some sympathomimetic amines in relation to monoamine oxidase. *Br. J. Pharmac. Chemother.*, **21**, 84–96.

ELTHERINGTON, L. G. & HORITA, A. (1960). Some pharmacological actions of *beta*-phenylisopropylhydrazine (PIH). *J. Pharmac. exp. Ther.*, **128**, 7–14.

EULER, U. S. VON & HELLNER-BJÖRKMAN, S. (1955). Effect of amine oxidase inhibitors on the noradrenaline and adrenaline content of cat organs. *Acta physiol. scand.*, **33**, Supp. 118, 21–25.

GOLDBERG, N. D. & SHIDEMAN, F. E. (1962). Species differences in the cardiac effects of a monoamine oxidase inhibitor. *J. Pharmac. exp. Ther.*, **136**, 142–151.

HOLTZ, P. & PALM, D. (1965). Über den mechanismus der sympathicomimetischen wirkungen einiger aliphatischer amine. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **252**, 144–158.

HORITA, A. (1963). The influence of drug-tissue interactions on the inhibition of monoamine oxidase by pheniprazine and iproniazid. *J. Pharmac. exp. Ther.*, **142**, 141–146.

HORITA, A. (1965). The initial inactivation of phenelzine by a monoamine oxidase-like system *in vitro* and *in vivo*. *Br. J. Pharmac. Chemother.*, **24**, 245–252.

LEE, W. C., SHIN, Y. H. & SHIDEMAN, F. E. (1961). Cardiac activities of several monoamine oxidase inhibitors. *J. Pharmac. exp. Ther.*, **133**, 180–185.

RYALL, R. W. (1961). Effects of cocaine and antidepressant drugs on the nictitating membrane of the cat. *Br. J. Pharmac. Chemother.*, **17**, 339–357.

SMITH, C. B. (1966). The role of monoamine oxidase in the intraneuronal metabolism of norepinephrine released by indirectly-acting sympathomimetic amines or by adrenergic nerve stimulation. *J. Pharmac. exp. Ther.*, **151**, 207–220.

TSAI, T. H. & FLEMING, W. W. (1965). Sympathomimetic actions of monoamine oxidase inhibitors in the isolating nictitating membrane of the cat. *Biochem. Pharmac.*, **14**, 369–371.

UDENFRIEND, S., WEISSBACH, H. & BRODIE, B. B. (1958). Assay of serotonin and related metabolites, enzymes and drugs. *Meth. Biochem. Anal.*, **6**, 95–130.