

Pharmacological Properties of Heterocyclic Amidine Derivatives. II.¹⁾
Pharmacological Studies of Phenylguanylpiperazine Derivatives²⁾

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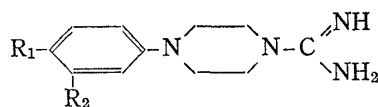
1-Phenyl-4-guanylpiperazine sulfate [PGP] and its derivatives were synthesized. The effects of these compounds on blood pressure, the nictitating membrane and the action of sympathomimetic amines such as tyramine and norepinephrine were examined in spinal cats. The effects of these compounds on MAO activity were also examined using the rat liver enzyme preparation *in vitro*. The results obtained were briefly summarized as follows:

- 1) PGP derivatives caused a rise in blood pressure and a contraction of the nictitating membrane in spinal cats.
- 2) PGP derivatives potentiated a pressor effect and a contraction of the nictitating membrane induced by tyramine and norepinephrine in spinal cats.
- 3) Reserpine caused a long-lasting hypertension and contraction of the nictitating membrane in spinal cats, when the animals were pretreated with PGP.
- 4) PGP derivatives showed a property to inhibit monoamine oxidase (MAO) activity.
- 5) PGP inhibited the restoration of the pressor effect of tyramine by a slow intravenous infusion of norepinephrine.

The pharmacological studies of amidine derivatives have been reported by many investigators for years. Recently a review concerned amidine derivatives was published by Fastier.⁴⁾ In the preceding paper, the authors¹⁾ reported the synthesis and the general pharmacological properties of amidine derivatives which were substituted with heterocyclic ring.

In the present paper the actions of 1-phenyl-4-guanylpiperazine (PGP) derivatives were reported on the nictitating membrane and the blood pressure of cats. Studies were also carried out on the influence of these compounds on the action of sympathomimetic amines, tyramine and norepinephrine. The mode of action of these compounds were also discussed.

TABLE I. Chemical Structure of Test Compounds



Cpds. No.	R ₁	R ₂	Formula	mp (decomp.) (°C)
I ^{a)}	H	H	C ₁₁ H ₁₆ N ₄ · ½H ₂ SO ₄ · H ₂ O	287—288
II	H	Cl	C ₁₁ H ₁₅ N ₄ Cl · ½H ₂ SO ₄	280
III	Cl	H	C ₁₁ H ₁₅ N ₄ Cl · ½H ₂ SO ₄	294
IV	H	CH ₃	C ₁₂ H ₁₈ N ₄ · ½H ₂ SO ₄	293—294
V	CH ₃	H	C ₁₂ H ₁₈ N ₄ · ½H ₂ SO ₄	298—300

a) 1-phenyl-4-guanylpiperazine (PGP)

- 1) Part I: H. Ozawa and K. Iwatsuki, *Yakugaku Zasshi*, **88**, 330 (1968).
- 2) The essentials of this paper were reported at the 41th Annual Meeting of the Japanese Pharmacological Society, Okayama, April 1968.
- 3) Location: No. 85, Kita-4-bancho, Sendai.
- 4) F.N. Fastier, *Pharmacol. Rev.*, **14**, 37 (1962).

Materials and Methods

The chemical structures of 1-phenyl-4-guanylpiperazine and its derivatives were shown in Table I.

Blood Pressure and Nictitating Membrane in Cats—Cats of both sexes, weighing 1.8 to 4.0 kg, were used. By Kumagai's method⁵⁾ spinal cats were prepared under ether anesthesia. The spinal cord was sectioned between C_I and C_{II}, and the brain was destroyed. The vagi were cut at both sides and the cats maintained under artificial respiration were left for one hour to recover from anesthesia. The left femoral vein was cannulated for the injection of drugs and the right femoral artery was also cannulated for the recording of blood pressure with a mercury manometer. Contraction of the nictitating membrane was recorded on a smoked paper. The compounds were dissolved in normal saline. All doses were expressed as weight of salts.

Inhibition of Monoamine Oxidase(MAO) *in Vitro*—Inhibition of monoamine oxidase (MAO) in the liver of rats was determined *in vitro* by the manometric technique.⁶⁾ Oxygen uptake was measured with a conventional Warburg apparatus. Rats, weighing 200 to 300 g, were sacrificed, then the liver was rapidly excised and a homogenate was prepared using an ice-cold isotonic solution of sucrose (0.25 M). The homogenate was centrifuged at about 0° at 700×g for 5 minutes in order to eliminate unbroken cells and tissue debris. The supernatant was centrifuged again at 16,000×g for 45 minutes under 0°.

The supernatant was discarded and the particles suspended in distilled water of equal weight of the liver was served as a source of the enzyme. Two-tenth ml of tyramine in a final concentration of 0.01 M was provided in the side arm as the substrate. The test system consisted of 0.2 ml of 0.3 M potassium cyanide and of 0.2 ml of 0.1 M semicarbazide and 0.2 ml of adequate concentrations of inhibitor. The final volume was adjusted to 2.0 ml with 1/15 M phosphate buffer solution (pH 7.4). The center well contained 0.1 ml of 20% KOH to absorb. CO₂.

The flasks were preincubated for 20 min at 37.5°. The substrate contained in the side arm was then tipped into the main compartment. Oxygen uptake was determined every 10 min for 90 min.

Results

Effect of PGP Derivatives on Blood Pressure and Response of the Nictitating Membrane in Spinal Cats

Intravenous injection of PGP in doses of 1–10 mg/kg immediately produced a rise in blood pressure and a prolonged contraction of the nictitating membrane in spinal cats. Blood pressure returned to normal within 15–60 min after the administration of the drug, but the contraction of the nictitating membrane persisted for two hours or more. Fig. 1 is a typical response to PGP. Another PGP derivative showed the same response.

Effect of PGP Derivatives on the Action of Tyramine and Norepinephrine in Spinal Cat

PGP derivatives, in a dose of 5 mg/kg, potentiated the pressor response and the contraction of the nictitating membrane induced by tyramine and norepinephrine in spinal cats, both in height and duration. These effects persisted for more than 4 hours. The results are shown in Table II and Table III. Fig. 2 and Fig. 3 show the typical responses of tyramine and norepinephrine before and after the administration of PGP (I) (5 mg/kg *i.v.*).

Effect of Reserpine on the Response of Blood Pressure and the Response of the Nictitating Membrane Pretreated with PGP

Fig. 4 shows the response elicited by reserpine (2 mg/kg *i.v.*) injected two hours after the administration of PGP (I) (5 mg/kg *i.v.*). Reserpine caused a long-lasting hypertension and

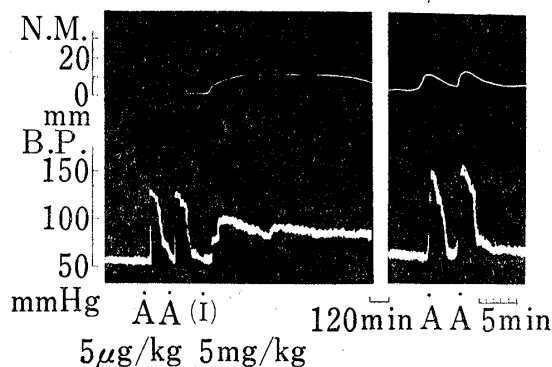


Fig. 1. Effect of PGP (I) on Blood Pressure and the Nictitating Membrane in the Spinal Cat

A: epinephrine 5 μg/kg *i.v.* (I) PGP 5 mg/kg *i.v.*
B.P.: blood pressure N.M.: nictitating membrane

5) M. Kumagai, T. Yui, K. Ogawa and H. Ohga, *Seitai no Kagaku*, 5, 132 (1953).

6) N.H. Creasey, *Biochem. J.*, 64, 178 (1956).

TABLE II. Effect of PGP Derivatives on the Action of Tyramine in the Spinal Cat

Compd. No.	B.P. ^{a)} mmHg		N.M. ^{b)} mm	
	before	after	before	after
I	116 ± 8.0	160 ± 4.6	4 ± 3.0	22 ± 5.8
II	131 ± 5.6	156 ± 5.5	1 ± 1.0	12 ± 4.2
III	110 ± 5.4	170 ± 7.2	2 ± 1.8	10 ± 4.2
IV	140 ± 4.8	168 ± 3.2	4 ± 1.5	18 ± 3.8
V	106 ± 6.6	170 ± 4.2	2 ± 1.0	21 ± 2.2

Test compounds were given intravenously in a single dose of 5 mg/kg.

B.P.: blood pressure N.M.: nictitating membrane

a) mean pressor response ± S.E. mmHg b) mean contractile response ± S.E. mm on the drum [tyramine (0.3 mg/kg)]

TABLE III. Effect of PGP Derivatives on the Action of Norepinephrine in the Spinal Cat

Compd. No.	B.P. ^{a)} mmHg		N.M. ^{b)} mm	
	before	after	before	after
I	104 ± 4.3	148 ± 5.2	3 ± 1.7	10 ± 2.1
II	96 ± 5.1	134 ± 4.8	1 ± 0.8	3 ± 1.1
III	100 ± 4.4	152 ± 5.3	1 ± 0.5	8 ± 3.0
IV	110 ± 2.2	130 ± 2.2	1 ± 0.4	6 ± 2.2
V	115 ± 4.8	151 ± 4.4	2 ± 0.3	4 ± 1.0

Test compounds were given intravenously in a single dose of 5 mg/kg.

B.P.: blood pressure N.M.: nictitating membrane

a) mean pressor response ± S.E. mmHg b) mean contractile response ± S.E. mmHg on the drum. [norepinephrine (1 µg/kg)]

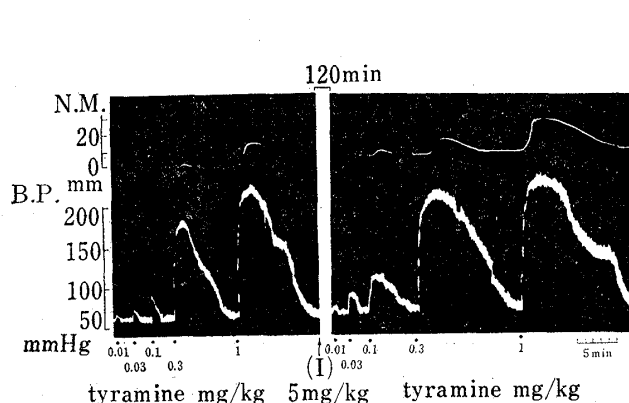


Fig. 2. A Typical Response of Tyramine in the Spinal Cat before and after (I) (5 mg/kg *i.v.*)

Tyramine was injected intravenously in doses of 0.01, 0.03, 0.1, 0.3, 1 mg/kg. (I) (5 mg/kg) was injected intravenously at the time signed by the arrow.

B.P.: blood pressure N.M.: nictitating membrane

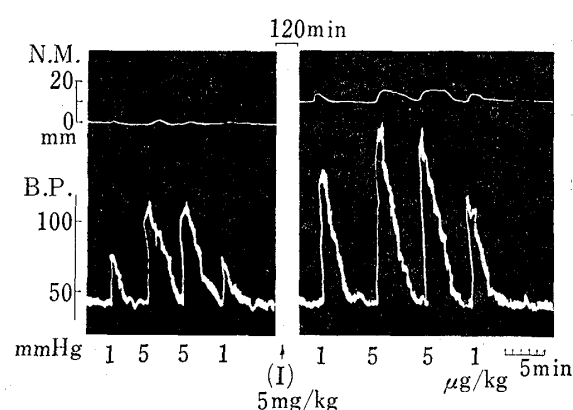


Fig. 3. A Typical Response of Norepinephrine in the Spinal Cat before and after (I) (5 mg/kg *i.v.*)

Norepinephrine was injected intravenously in doses of 1 and 5 µg/kg. 5 mg/kg of (I) was injected intravenously at the time signed by the arrow.

B.P.: blood pressure N.M.: nictitating membrane

contraction of the nictitating membrane, which persisted for three hours or more. The latter was just like a stimulation of the sympathetic nerve and was antagonized by an α -blocking agent, phentolamine (5 mg/kg *i.v.*).

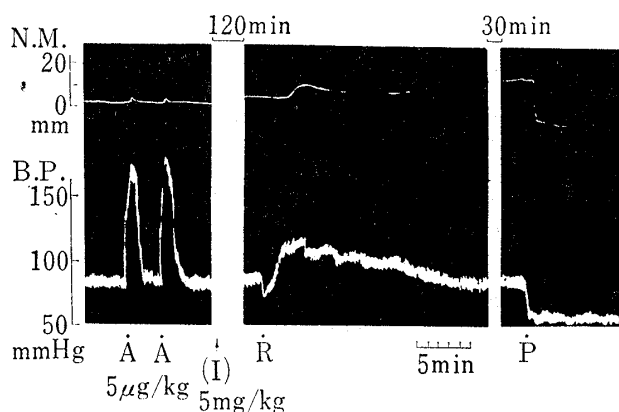


Fig. 4. Effect of PGP (I) on the Responses of Blood Pressure and the Nictitating Membrane induced by an Intravenous Injection of Reserpine in the Spinal Cat

A: epinephrine 5 μ g/kg *i.v.* arrow: PGP (I) 5 mg/kg *i.v.*
 R: reserpine 2 mg/kg *i.v.* P: phentolamine 5 mg/kg *i.v.*
 B.P.: blood pressure N.M.: nictitating membrane

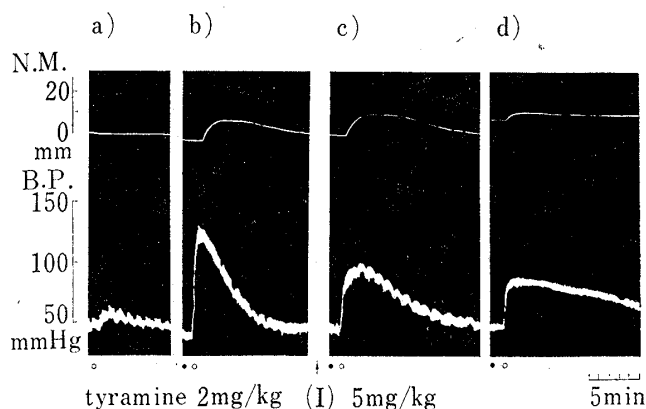


Fig. 5. Effect of PGP (I) on the Action of Tyramine in a Cat previously treated with Reserpine

The cat was treated with reserpine (2 mg/kg subcutaneously) 48 hours previously.

open circles: tyramine 2 mg/kg *i.v.*

closed circles: end of norepinephrine infusion, at a rate of 40 μ g/min for 10 min

Between b and c PGP (I), 5 mg/kg, was administered intravenously.

B.P.: blood pressure N.M.: nictitating membrane

Effect of PGP on the Action of Tyramine in Cats Previously Treated with Reserpine

Fig. 5 shows a response of blood pressure induced by tyramine in a spinal cat previously treated with reserpine. The cat was pretreated with reserpine (2 mg/kg) subcutaneously 48 hours before the experiment. Norepinephrine (20 μ g/ml *i.v.*) was infused at a rate of 2 ml/min for 10 min in order to restore the response to tyramine on blood pressure. As shown in Fig. 5, a rise in blood pressure by tyramine (2 mg/kg *i.v.*) was less than 10 mmHg before (a), but it was about 100 mmHg after the infusion of norepinephrine (b). If PGP (I) (5 mg/kg *i.v.*) was injected, the responses induced by tyramine radically changed (c,d) and the rise in blood pressure by tyramine was depressed as compared with (b).

Inhibition of MAO *in Vitro*

The data in Table IV show the inhibition by PGP derivatives and nialamide at various concentrations of oxygen uptake by rat liver enzyme *in vitro*. As shown in the table, all of PGP and its derivatives exerted an inhibition of MAO activity. The compound which showed the most potent MAO inhibition was compound III. And it caused 53.2% inhibition of MAO at the concentration of 5×10^{-4} M. The degree of MAO inhibition was almost the same as that of nialamide.

TABLE IV. Inhibition of Monoamine Oxidase (MAO) *in Vitro*

Compounds No.	Inhibition (%)		
	5×10^{-4} M	5×10^{-5} M	5×10^{-6} M
I	44.2	9.1	0
II	49.5	9.8	0
III	53.2	10.6	0
IV	44.8	5.9	0
V	37.3	4.5	0
Nialamide	61.9	13.2	0

The activity of MAO inhibition was determined 90 minutes after the beginning of incubation. All values are the average of at least six determinations.

Discussion

The intravenous injection of PGP and its derivatives produced a sympathomimetic effect, which was shown by a rise in blood pressure and a prolonged contraction of the nictitating membrane (Fig. 1).

PGP derivatives strongly potentiated the pressor effect and the contraction of the nictitating membrane induced by tyramine (Fig. 2 and Table II), both in height and duration. As the reason for this potentiation, the inhibition of MAO activity by these compounds can be considered. When the animals are treated with MAO inhibitors, tyramine itself and norepinephrine released by tyramine are not metabolized and exerts more potent activity, consequently resulting in a potentiation of the action of tyramine. Griesemer⁷⁾ and Rebhurn⁸⁾ reported that iproniazid potentiated the action of tyramine.

It is well known that reserpine can induce hypertension in animals pretreated with MAO inhibitors,^{9,10)} cocaine,¹¹⁾ and ephedrine or other sympathomimetic amines.^{9,12)} As shown in Fig. 4, the intravenous administration of reserpine (2 mg/kg) in the spinal cat two hours after PGP (I) (5 mg/kg) produced a very marked and long-lasting hypertension and contraction of the nictitating membrane. The latter was antagonized by α -blocking agent, phentolamine (2 mg/kg).

There are currently differences of opinions as to the mechanism of this hypertension. Chessin¹⁰⁾ has suggested that a release of serotonin in the central nervous system can be responsible for such hypertension, but N,N-diethyl lysergamide (LSD 25), a serotonin antagonist, does not antagonize the pressor effect of reserpine.^{12,13)} The hypertension induced by reserpine would be more likely explained by a peripheral release of sympathetic amine (norepinephrine) which was destroyed by MAO. When MAO is inhibited, norepinephrine released by reserpine may remain at its site of release, reach the receptor site directly and cause hypertension.

From our experiments in spinal cats it is suggested that PGP derivatives have a property to inhibit MAO activity. Therefore, the inhibition by these compounds of MAO activity were examined *in vitro*. The results obtained indicate that all of PGP derivatives shows the inhibition of MAO activity as potent as nialamide (Table IV). Although most of MAO inhibitors are chemically hydrazine derivatives, piperazine derivatives substituted with amidino group were found to have an activity of MAO inhibition. Introduction of halogen and methyl group into phenyl ring has a slight influence on the potency of MAO inhibitory activity.

PGP derivatives also potentiated the action of norepinephrine upon blood pressure as much as tyramine (Fig. 3 and Table III). Iproniazid showed no effect on the action of norepinephrine injected exogenously,¹⁴⁾ but nialamide potentiate the action of norepinephrine.¹⁵⁾ From these points, the property of these compounds is not identical with that of iproniazid.

As shown in Fig. 5, PGP (I) (5 mg/kg) inhibited the restoration of norepinephrine. The dose of tyramine, 2 mg/kg, is sufficient to cause a pressor response (Fig. 2) but such an action of tyramine was almost depressed in the spinal cat pretreated with reserpine. This shows that norepinephrine at its store site was completely depleted by reserpine (Fig. 5, a). After norepinephrine infusion the pressor response was almost recovered (Fig. 5, b). Burn and

- 7) E. C. Griesemer, J. Barsky, C. A. Welles and E. A. Zeller, *Proc. Soc. Exptl. Biol. Med.*, **84**, 699 (1953).
- 8) J. Rebhurn, S.M. Geinberg and E.A. Zeller, *Proc. Soc. Exptl. Biol. Med.*, **85**, 218(1954).
- 9) M. Chessin, B. Dubnick, G. Leeson and C.C. Scott, *Ann. N.Y. Acad. Sci.*, **80**, 597 (1959).
- 10) M. Chessin, E.R. Kramer and C.C. Scott, *J. Pharmacol. Exptl. Therap.*, **119**, 453(1957).
- 11) F.G. Valdecasas, J.A. Salva and E. Cuenca, *Arzneimittelforsch.*, **8**, 655(1958).
- 12) H. Schmitt and H. Schmitt, *Arch. Intern. Pharmacol.*, **120**, 251(1959).
- 13) A.R. Maxwell, S.D. Ross, A.J. Plumma and E.B. Sigg, *J. Pharmacol. Exptl. Therap.*, **119**, 69 (1957).
- 14) S. Garattini, P. Fresia, A. Mortari and V. Palma, *Med. Exp.*, **2**, 252(1960).
- 15) M.J. Davey, J.B. Farmer and H. Reiner, *Brit. J. Pharmacol.*, **20**, 121(1963).

Rand¹⁶⁾ also reported that these effects of tyramine could be restored by slow intravenous injection of norepinephrine. The fact that the response of tyramine was not recovered by norepinephrine infusion after PGP (I) (5 mg/kg) (Fig. 5, c, d) suggests that this agent interferes with the restoration of norepinephrine in its storage site. This seems to be responsible for the potentiation of the action of norepinephrine on blood pressure and the nictitating membrane in spinal cats. The same result was obtained using nialamide by Davey.¹⁵⁾

From these results, it is concluded that the potentiation of the action of tyramine and norepinephrine by PGP derivatives is due to the inhibition of MAO activity and of the restoration of norepinephrine. But the possibilities would not be excluded that PGP derivatives alter directly the sensitivity of the receptor to norepinephrine or there exist other unknown mechanisms.

16) J.H. Burn and M.J. Rand, *J. Physiol.*, **144**, 341 (1958).