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104. Hikaru Ozawa and Susumu Sato : The Adrenergic Neurone Blocking Action of [2-(Methylphenylamino)-ethyl]guanidine Sulfate. II.*1

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[2-(Methylphenylamino)ethyl]guanidine sulfate (MPG) was found to be an effective antihypertensive agent during repeated administration at 10 mg./kg. subcutaneously to conscious renal hypertensive rats. Prolonged reduction of contractions evoked by preganglionic stimulation of the cervical sympathetic nerve in the nictitating membrane of cat was observed following a single intravenous administration of 10 mg./kg. of MPG. This procedure also caused the potentiation of norepinephrine-induced responses and suppression of tyramine- or amphetamine-induced response in the nictitating membrane of the cat and blood pressure of the dog. In the experiment of intra-arterial administration, the postganglionic cervical sympathetic nerve blocking activity was observed to be one-half as potent as guanethidine in the nictitating membrane of cat. The hypotensive effect of MPG would be considered to be depletion of catecholamines and blockade of sympathetic transmission at adrenergic nerve level.

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In a recent study,*1 it was demonstrated that the pharmacological properties of [2-(methylphenylamino)ethyl]guanidine sulfate (MPG) resembled qualitatively to that of guanethidine in acute experiment. Both compounds inhibited the efferent sympathetic transmission in many preparations, suppressed the tyramine-induced hypertension and potentiated the catechoamine-induced hypertension. The inhibitory effect of MPG on the excitatory response evoked by electrical stimulation of the peripheral sympathetic nerves was abolished by amphetamine and this suggested that the adrenergic blocking action of MPG was depend upon the same mechanism as guanethidine and bretylium.[1]

In this report, the authors directed their attention toward (1) the determination of the antihypertensive effect of MPG on the conscious renal hypertensive rat, and (2) duration of adrenergic neurone blocking effect after a single intravenous administration. The study of adrenergic blocking action was also carried out in the nictitating membrane of cat using the method of intra-arterial administration to the postganglionic nerve and nictitating membrane.

Experimental Methods

For the preparation of a renal hypertensive rat, the method developed by Kempf, *et al.*[2] was used. The operation was performed in two stages. The first stage consisted of the unilateral nephrectomy. In the second stage, one week after the first operation, the branch of the renal artery which supplies the anterior portion of contralaberal kidney was ligated.

The method developed by Byrom, *et al.*[3] was used for measuring the systolic blood pressure of the intact, conscious rat using an air pressure cuff and prethysmograph on the tail. The rat was warmed for 10 minutes in a box kept 38 to 40°, placed in the holder and the tail was inserted into the plethysmograph.

To study the effect on the nictitating membrane of cats, the cats were anesthetized with urethane (1.4 g./kg., *s.c.*) and the cervical sympathetic chain was stimulated preganglionically with supramaximal rectangular pulses (0.7 msec. duration, 1~20 shocks/sec. for 15 sec.), using Nihon Koden MSE-20 stimulator.

*1 Part I : This Bulletin, **14**, 1291 (1966). This work was reported Tohoku Branch Meeting of the Pharmaceutical Society of Japan, Sendai, July, 1966.

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1) M. D. Day : Brit. J. Pharmacol., **18**, 421 (1962).
2) G. H. Kempf, I. H. Page : J. Lab. Clin. Med., **27**, 1192 (1942).
3) F. B. Byrom, C. Willson : J. Physiol. (London), **93**, 301 (1938).

Contractions of the nictitating membrane were recorded with an isotonic lever system on kymograph. The same magnification was employed in all studies.

For intra-arterial administration, the method developed by Morrison, *et al.*[4] and by Trendelenburg[5] was used. A polyethylene cannula was inserted retrogradely into the lingual artery, so that its tip lies at the junction with the external carotid artery and drugs passed only to the nictitating membrane and post-ganglionic nerve.

Five dogs of both sexes were anesthetized with sodium pentobarbital (40 mg./kg., *i. v.*) and arterial blood pressure was recorded with Nihon Koden RM-150 electromanometer following direct cannulation of the femoral artery.

The following drugs were dissolved in 0.9 w/v % of aqueous sodium chloride :

[2-(Methylphenylamino)ethyl]guanidine sulfate (MPG), [2-(octahydro-1-azocinyl)ethyl]guanidine sulfate (guanethidine), *dl*-norepinephrine hydrochloride, tyramine hydrochloride and *dl*-amphetamine sulfate. All doses are expressed as weights of salts.

Results

Antihypertensive Effect in Renal Hypertensive Rats

The antihypertensive activity of MPG following subcutaneous administration was studied in conscious hypertensive rats. Four rats received MPG at 10 mg./kg. subcutaneously for 14 days. The drug gradually produced a fall in blood pressure following subsequent administration. The fall in blood pressure reached a maximum within 10 days and returned to the pre-dose level 1 week after withdrawal of the drug. The results were shown in Fig. 1.

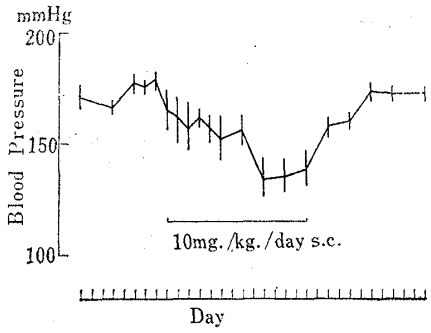

Fig. 1. Blood Pressure Lowering Action of MPG (10 mg./kg./day *s. c.*) in Male Rats with Renal Hypertension

Mean values of 4 animals. Vertical bars represent standard errors of mean.

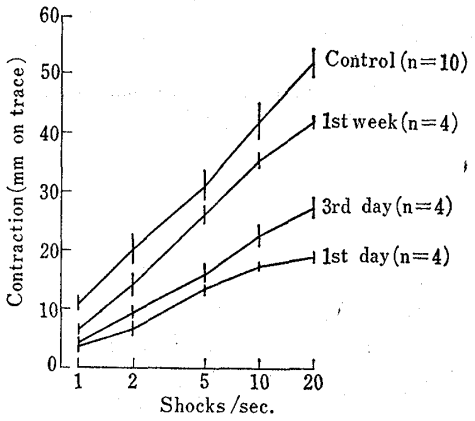

Fig. 2. Mean Heights of Contraction of the Nictitating Membrane of Urethane Anesthetized Cats by Stimulation of the Preganglionic Cervical Sympathetic Nerve at Various Frequencies for 15 sec. Periods.

Contractions are examined for 10 controls, for 4 cats at the 1st day, for 4 cats at the 3rd day and 4 Cats at the 1st week after a single intravenous administration of MPG respectively. The figures in brackets indicate the number of cats is the group. Verical bars represent standard errors of mean.

Study on the Nictitating Membrane of Cats

A single intravenous administration of 10 mg./kg. of MPG on conscious 8 cats brought about moderate relaxation of the nictitating membrane after a latent period of 6 to 15 hours. This relaxation was still prominent at 48 and 72 hours post administration.

In 12 cats a single intravenous administration of 10 mg./kg. of MPG produced the inhibition of the contractions of the nictitating membrane on the stimulation of the

4) B. Morrison, W. D. M. Paton : Brit. Med. J., **1**, 1299 (1953).
5) U. Trendelenburg : Fed. Proc., **18**, 1001 (1959).

preganglionic cervical sympathetic nerve. Intravenous administration of MPG brought about the marked to the moderate inhibition of the nictitating membrane at the 1st and 3rd day in 8 cats. Slight inhibition was observed 1 week after MPG in 4 cats. The results of this experiment were summarized in Fig. 2.

In 13 cats, the effect of MPG in a single intravenous administration of 10 mg./kg. on the contractions of the nictitating membrane evoked by intravenous injection of norepinephrine and tyramine were also examined. Contractions elicited by the intravenous administration of 5 μg./kg. of norepinephrine was markedly potentiated at the 1st and 3rd day after MPG. This potentiation was declined 1 week after MPG. MPG also markedly suppressed the contraction elicited by the intravenous administration of 0.5 mg./kg. of tyramine. Marked suppression was observed at the 1st and 3rd day after MPG. The results were summarized in Table I.

Table I. Effect of MPG on the Response of Norepinephrine and Tyramine in the Nictitating Membrane of Urethane Anesthetized Cats

Amine (Dose. *i.v.*)	Untreated Control	Mean contractile response ± S.E. mm after MPG (10 mg./kg., *i.v.*)		
		1st day	3rd day	1st week
Norepinephrine (5 μg./kg.)	1.00±0.42 (7)	16.0 ±1.04 (5)	18.0 ±3.44 (4)	3.30±2.85 (4)
Tyramine (0.5 mg./kg.)	14.0 ±2.63 (7)	3.60±1.17 (5)	8.00±2.00 (4)	4.30±2.85 (3)

MPG was given intravenously in a single dose of 10 mg./kg.
( ): Number of cats

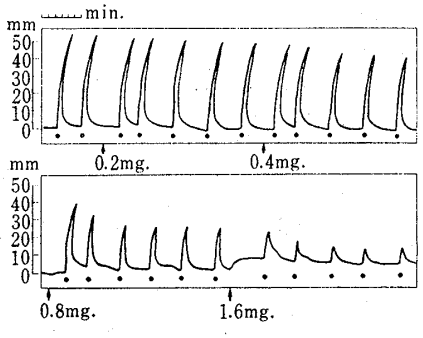

Fig. 3. Effect of MPG administered Intra-arterially to the Nictitating Membrane and Postganglionic Nerve on the Contractions of the Nictitating Membrane of Urethane Anesthetized Cats by Stimulation of the Preganglionic Cervical Sympathetic Nerve (rectangular impulses of 0.7 msec. duration, frequency 20 shocks/sec., period of stimulation 15 sec.)
Electrical stimulation was given at dots.

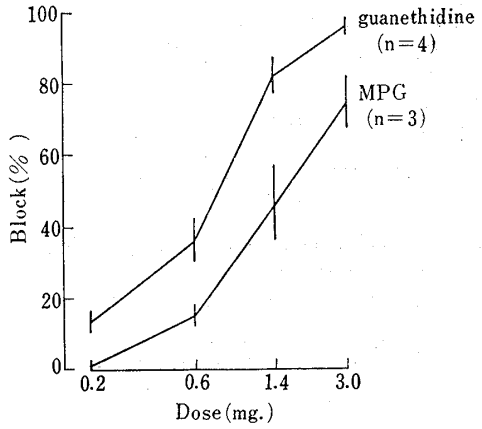

Fig. 4. Inhibition of Contractions of the Nictitating Membrane of Cats by Intra-arterially Injected MPG and Guanethidine following Preganglionic Stimulation (rectangular impulses of 0.7 msec. duration, frequency 20 shocks/sec., period of stimulation 15 sec.)

Abscissa: dose in mg. i.a.
Ordinate: Inhibition of contraction of the nictitating membrane expressed in % of the initial value. Vertical bars represent the standard errors of mean. The figures in brackets indicate the number of cats in the group.

The intra-arterial administration of MPG or guanethidine to the nictitating membrane and postganglionic nerve inhibited the contractions of the nictitating

membrane on stimulation of preganglionic cervical sympathetic nerve. Both drugs in relatively high doses produced small contraction of the membrane. The typical example was shown in Fig. 3. By this experimental procedure, the potency of MPG in depressing transmission of postganglionic nerve was estimated as about 1.5 mg. in 50% of the effective dose and one-half as potent as guanethidine. Fig. 4 showed the dose-response curves obtained from the experiment using the technique illustrated in Fig. 3.

Inhibition of Tyramine- and Amphetamine-induced Hypertension in Dogs

A single intravenous administration of 10 mg./kg. of MPG in 5 anesthetized dogs suppressed the pressor response elicited by the intravenous administration of 0.3 or 0.5 mg./kg. of tyramine. Marked suppression was observed at the 1st and 3rd day after MPG administration. The suppression continued beyond 1 week after single injection but could not be observed at 2 and 4 weeks. An intravenous administration of 10 mg./kg. of MPG similarly suppressed the pressor response produced by the intravenous administration of 0.5 mg./kg. of amphetamine in 5 dogs. This inhibitory effect on amphetamine-induced hypertension reached its maximum at the 1st day and was still present 2 weeks after a single administration of MPG. The typical examples were shown in Fig. 5.

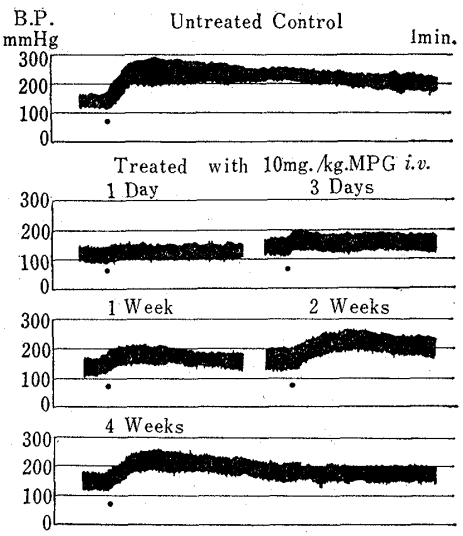

Fig. 5. Time Course of Inhibition of Amphetamine-induced Hypertension by MPG in the Anesthetized Dog

Time notations refer to time elapsed after a single intravenous injection of MPG. 0.5 mg./kg. of amphetamine was administered intravenously at dots. All tracing are from the same animal.

Potentiation of the Norepinephrine-induced Hypertension in Dogs

A single intravenous administration of 10 mg./kg. of MPG profoundly enhanced the pressor response elicited by the intravenous administation of 3 or 5 μg./kg. of norepinephrine in 5 anesthetized dogs. One day after pretreatment with 10 mg./kg. of MPG, the pressor responses of norepinephrine were double those generally

Table II. Effect of MPG on Norepinephrine-, Tyramine- and Amphetamine-induced Hypertension in 5 Anesthetized Dogs

Amine (Dose. *i.v.*)	Untreated Control	Mean pressor response ± S.E. mm.Hg after MPG (10 mg./kg., *i.v.*)				
		1st day	3rd day	1st week	2nd week	4th week
N.E.						
(3 μg./kg.)	55.6±16.1	110.6±16.1	97.6±11.1	75.6±10.3	70.4± 6.12	75.6± 8.52
(5 μg./kg.)	65.0±16.3	132.2±18.4	116.3±14.4	87.3±13.9	81.0±13.0	80.6± 6.53
Tyr.						
(0.3 mg./kg.)	84.8±14.9	25.2± 6.28	33.6± 5.28	51.8± 9.61	78.2±13.1	86.6±13.9
(0.5 mg./kg.)	100.8±14.2	33.6± 5.60	38.6± 7.81	71.8±10.8	99.6± 9.93	104.6±14.3
Am.						
(0.5 mg./kg.)	67.6± 7.08	12.0± 1.22	17.6± 2.85	36.4± 2.60	47.6± 4.69	61.6± 6.84

MPG was given intravenously in a single doses of 10 mg./kg.
N.E.: norepinephrine Tyr.: Tyramine Am.: amphetamine

observed before treatment. Partial potentiation lasted for even 4 weeks after MPG. The effects of MPG on norepinephrine-, tyramine-and amphetamine-induced hypertension in dogs were summarized in Table II.

Discussion

The studies of MPG in a single administration on the nictitating membrane of cat indicated that MPG interfered with the release of transmitter substances from adrenergic nerve stores for at least 1 week. At the same time, the different actions of MPG on sympathomimetic amines, *i.e.*, potentiation of norepinephrine-induced response and suppression of tyramine- or amphetamine-induced response, were observed. The adrenergic neurone blocking action of MPG accompanied with the different action on sympathomimetic amines is analogous to those reported after the administration of reserpine[6] or guanethidine,[7] which can cause a depletion of endogenous catecholamines from adrenergic nerve stores.[8~11]

Assuming that MPG could deplete catecholamines as reserpine or guanethidine did, although there is no direct evidence on this point, it would be explainable that MPG suppressed the tyramine- and amphetamine-induced responses which are elicited by the liberation of catecholamines from adrenergic nerve stores.[12,13] But the possibility of bretylium-like effect, which can produce blockade of sympathetic system without amine depletion,[14] was not excluded in accute experiment.

Therefore, the antihypertensive effect of MPG on the renal hypertensive rat might be mainly depend upon the depletion of catecholamines from adrenergic nerve stores.

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