

239. Hikaru Ozawa and Isao Otsuki*¹: Pharmacological Studies
on Guanethidine Derivatives. V.*² Adrenergic Neurone
Blocking Action of 2,3,4,5-Tetrahydro-1H-2-benz-
azepine-2-carboxamide Sulfate.

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Pharmacological properties of 2,3,4,5-tetrahydro-1H-2-benzazepine-2-carboxamide sulfate (GQ-1) has been studied mainly on sympathetic nervous system and the following results were obtained: 1) GQ-1 showed the adrenergic neurone blocking activity three-fifth as potent as that of guanethidine in urethane anesthetized cat nictitating membrane. 2) Unlike with the cases of guanethidine and guanisoquin, GQ-1 did not cause the contraction of the nictitating membrane nor did the rise of the blood pressure in urethane anesthetized cat. 3) GQ-1 potentiated the pressor response to norepinephrine and markedly suppressed the pressor response to tyramine in cat.

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In 1960 guanethidine was introduced by Maxwell, *et al.*,¹⁾ as an adrenergic neurone blocking agent and it has proved to be of value in management of hypertension in clinic.²⁾ Later it became evident that guanethidine depleted the catecholamine content of adrenergic nerve endings.³⁾

Recently guanisoquin, a new adrenergic neurone blocking agent, has been reported by Scriabine, *et al.*⁴⁾: it is a derivative of tetrahydroisoquinoline. The authors have already reported the pharmacological properties of some guanethidine derivatives.^{5~7)}

The present paper is concerned with the pharmacological properties of 2,3,4,5-tetrahydro-1H-2-benzazepine-2-carboxamide sulfate (GQ-1), it is related chemically to both guanethidine and guanisoquin. The structural formula of GQ-1 is shown in Chart 1 with guanethidine and guanisoquin for comparison.

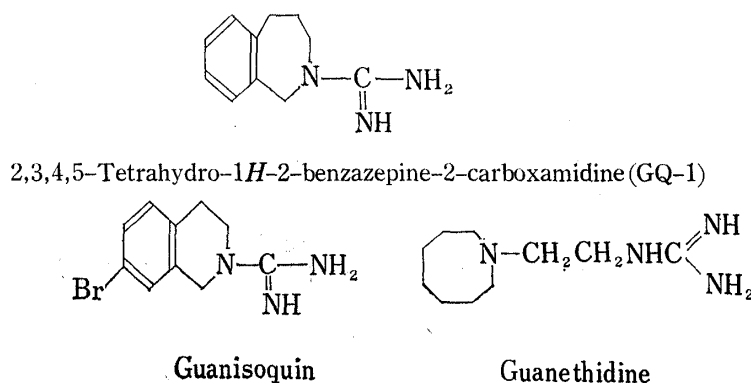


Chart 1.

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*² Part III. H. Ozawa, Y. Gomi, I. Otsuki: *Yakugaku Zasshi* **85**, 112 (1965). This work was reported at the North Area Regional Meeting of the Japanese Pharmacological Society, Oct. 2, 1966.

1) R.A. Maxwell, A.J. Plummer, H. Povalski, A.J. Daniel: *J. Pharmacol. Exptl Therap.*, **128**, 22 (1960).

2) D.W. Richardson, E.M. Wyso: *Ann. N.Y. Acad. Sci.*, **88**, 944 (1960).

3) R. Cass, R. Kuntzman, B.B. Brodie: *Proc. Soc. Exptl. Biol. Med.*, **103**, 871 (1960).

4) A. Scriabine, K.D. Booher, J.N. Pereira, W.K. McShane, J.W. Constantine, R.C. Koch, S. Miknius: *J. Pharmacol. Exptl. Therap.*, **147**, 277 (1965).

5) H. Ozawa, H. Fukuda, I. Otsuki: *Yakugaku Zasshi*, **83**, 629 (1963).

6) H. Ozawa, I. Otsuki: *Ibid.*, **84**, 67 (1964).

7) H. Ozawa, Y. Gomi, I. Otsuki: *Ibid.*, **85**, 112 (1965).

Methods

The chemical formula of GQ-1 is $C_{11}H_{15}N_3 \cdot 1/2H_2SO_4$; its molecular weight is 237.0. It is a white crystalline powder.

To study the effect on the blood pressure and the nictitating membrane, cats both sexes were anesthetized with urethane 1.4~1.6 g./kg. by subcutaneous injection.

The blood pressure was recorded from the carotid artery or femoral artery on kymograph. The cervical sympathetic nerve and the greater splanchnic nerve were stimulated with supramaximal rectangular pulses using Nihon Kodon MSE-20 stimulator.

Contractions of the nictitating membrane were recorded with an isotonic lever system on kymograph. The same magnification was employed in all studies. The vagolytic activity was studied in the dog anesthetized with sodium pentobarbital 30 mg./kg. by intravenous injection and the blood pressure in the carotid artery was recorded with electromanometer. The peripheral end of the left vagus was stimulated with rectangular pulses. The injections were usually made into the femoral vein.

To study the effect on the isolated intestine, the method developed by Finkleman (1930)⁸⁾ was used. The mesenteric nerve was stimulated through the platinum electrode with supramaximal voltage at 50 shocks/sec. for 20 seconds. Actions at the neuromuscular junction were tested on the rat diaphragm-phrenic nerve preparation by the method of Bülbring (1946).⁹⁾ Rectangular pulses of supramaximal voltage of 1~4 msec. duration were applied to the nerve through platinum electrode. Guinea-pig isolated heart was perfused by the method of Langendorff. Tyrode solution was used for the rabbit intestine, Krebs-Henseleit solution for rat diaphragm and guinea-pig heart. These solutions were oxygenated and maintained at 37°.

The following drugs were used; 2,3,4,5-tetrahydro-1*H*-2-benzazepine-2-carboxamide sulfate (GQ-1), [2-(octahydro-1-azocinyl)ethyl]guanidine sulfate (guanethidine), 1-benzyl-1-methyl-3-methyl guanidine sulfate (bethanidine), *dl*-norepinephrine hydrochloride, tyramine hydrochloride, *dl*-amphetamine sulfate.

All doses were expressed as weight of salts.

Results

Effects of GQ-1 on the Nictitating Membrane of the Unanesthetized Cats

In the cat, subcutaneous injection of 10 mg./kg. of GQ-1 caused maximal relaxation of the nictitating membrane after the latent period of 2 hours. The effect was the greatest between 6 to 12 hours. The tone of the nictitating membrane returned to normal after 2 days.

An example is shown in Fig. 1.

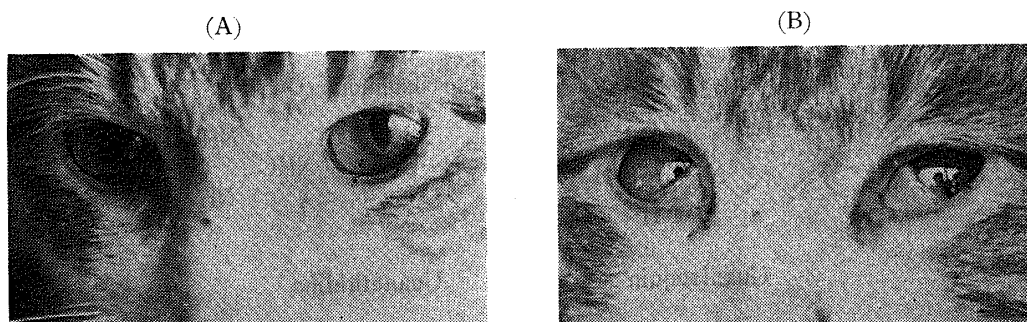


Fig. 1. Effect of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Nictitating Membrane of the Unanesthetized Cat(3.0 kg., male)
(A) before experiment (B) six hours after the subcutaneous injection of 10 mg./kg. of GQ-1.

Effects of GQ-1 on the Nictitating Membrane of the Urethane Anesthetized Cats

As shown in Fig. 2, intravenous injection of 5 mg./kg. of GQ-1 in the cat resulted in an inhibition of the contraction of the nictitating membrane elicited by the stimulation

8) B. Finkleman : J. Physiol. (Lond.), **70**, 145 (1930).

9) E. Bülbring : Brit. J. Pharmacol., **1**, 38 (1946).

of the pre- and post-ganglionic cervical sympathetic nerve. No contraction of the nictitating membrane following the intravenous injection of GQ-1 was observed, which therefore differs in this respect from guanethidine and guanisoquin that produce a sustained contraction.

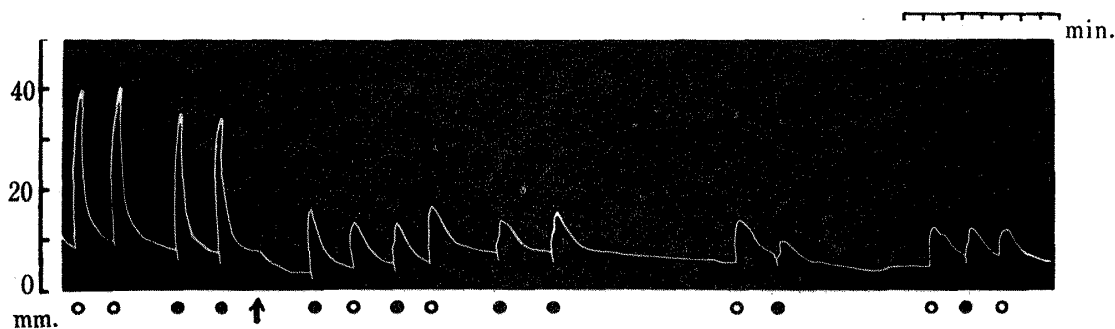


Fig. 2. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Contractions of the Nictitating Membrane elicited by Pre- and Post-ganglionic Stimulation of the Cervical Sympathetic Nerve of a Cat Anesthetized with Urethane.

empty circles: cervical sympathetic preganglionic stimulation at 20 c.p.s., 0.7 msec., 3 volt for 15 seconds.

filled circles: cervical sympathetic postganglionic stimulation at 20 c.p.s., 0.7 msec., 15 volt for 15 seconds. At the arrow GQ-1 (5 mg./kg.) was given intravenously.

Intravenous injection of 1 mg./kg. of GQ-1 resulted in an inhibition of the contraction of the nictitating membrane which was elicited by preganglionic electrical stimulation at various frequencies (0.5~20 c.p.s., 0.7 msec., supramaximal voltage for 15 seconds) to the cervical sympathetic nerve. The response of the nictitating membrane to the stimulation of the sympathetic nerve was reduced almost completely after the administration of 5 mg./kg. of GQ-1. These reduced responses were recovered almost completely following the intravenous administration of 1 mg./kg. of amphetamine. See the frame of (D) and (E) in Fig. 3. Even 24 hours after 10 mg./kg. of GQ-1 by intravenous injection, the response of the nictitating membrane to the stimulation of the sympathetic nerve was reduced.

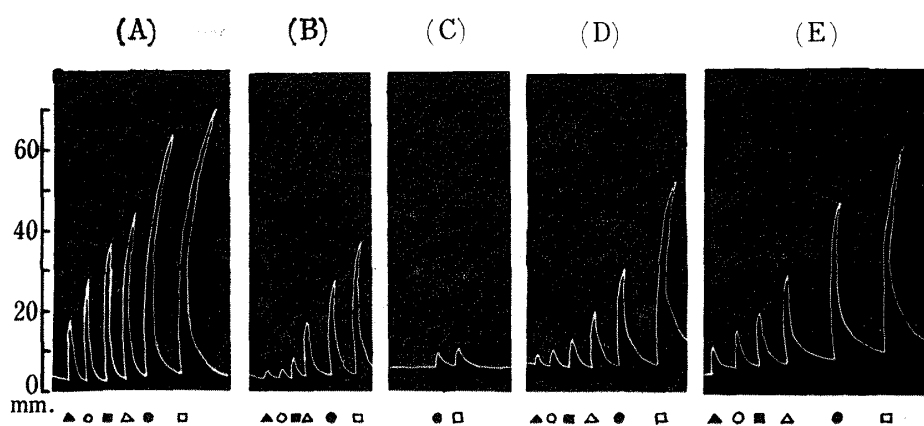


Fig. 3. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate (GQ-1) on the Contractile Responses of the Nictitating Membrane elicited by Preganglionic Sympathetic Nerve Stimulation in a Urethane Anesthetized Cat.

Preganglionic nerve was stimulated with rectangular pulse of supramaximal voltage 0.7 msec. duration, at various frequencies (0.5~20 shocks/sec.).

(A): control responses

(B): 30 min. after 1 mg./kg. of GQ-1 intravenously.

(C): 30 min. after 5 mg./kg. of GQ-1 intravenously.

(D): one hour after 1 mg./kg. of amphetamine intravenously.

(E): three hours after 1 mg./kg. of amphetamine intravenously.

Filled triangles: 0.5 c.p.s., Empty circles: 1 c.p.s.,

Filled squares: 2 c.p.s., Empty triangles: 5 c.p.s.,

Filled circles: 10 c.p.s., Empty squares: 20 c.p.s.

As shown in Fig. 4, contractions of the nictitating membrane produced by the intravenous injection of 5 μ g./kg. of norepinephrine was slightly potentiated by the 1 to 5 mg./kg. of GQ-1. GQ-1 also markedly diminished the contraction produced by the intravenous injection of 1 mg./kg. of tyramine.

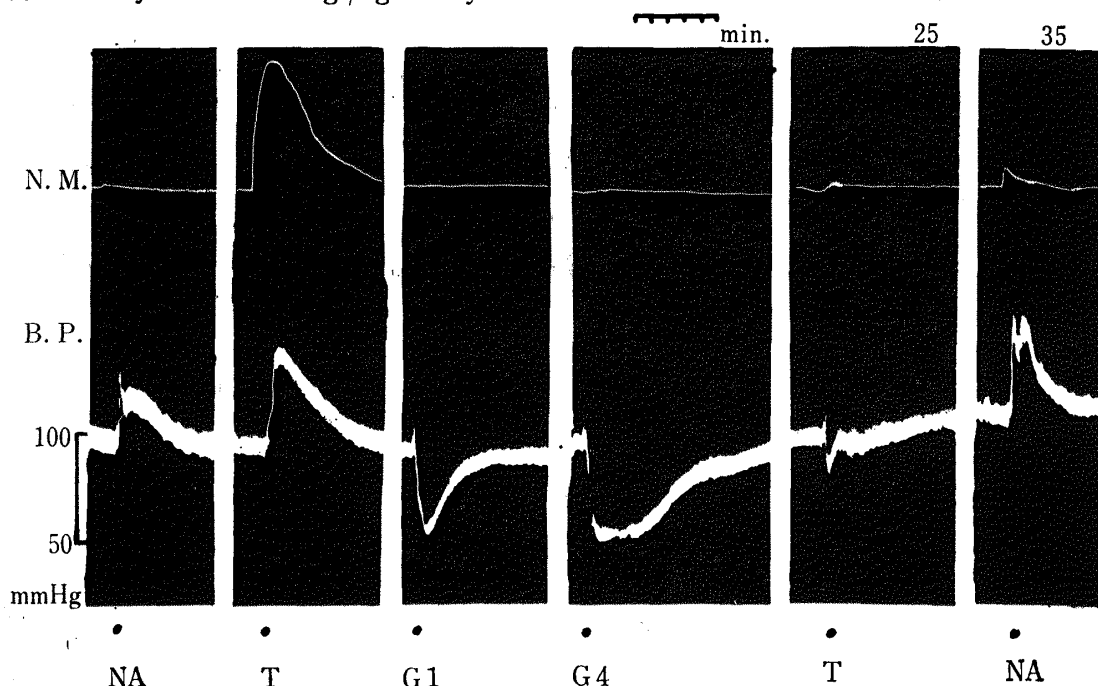


Fig. 4. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Nictitating Membrane (upper record) and the Blood Pressure (lower record) of a Cat anesthetized with Urethane

Norepinephrine (5 μ g./kg.) was injected intravenously at NA and 1 mg./kg. of tyramine at T. GQ-1 was injected intravenously, 1 mg./kg. at G1 and 4 mg./kg. at G4.

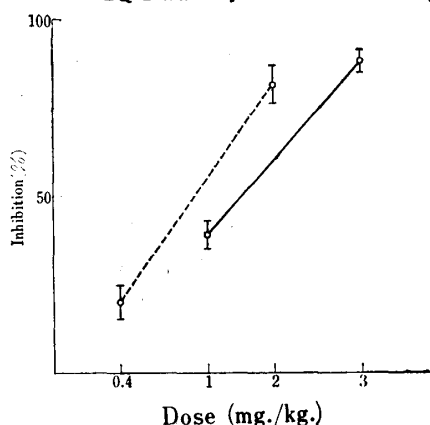


Fig. 5. Dose-Inhibition Curves of Guanethidine and 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) for the Contractile Responses of the Nictitating Membrane elicited by Preganglionic Supramaximal Electrical Stimulation(0.7 msec., 20 c.p.s., for 15 sec.) of the Cervical Sympathetic Nerve in Urethane Anesthetized Cats

The vertical bars on each dose-inhibition curves represent the standard errors of means.

Abscissa: dose in mg./kg. injected intravenously.

----- Guanethidine
 ———— GQ-1

Ordinate: Inhibition per cent of contraction of the nictitating membrane.

An attempt was made to evaluate guanethidine and GQ-1 quantitatively in their effects on the contractions of the nictitating membrane elicited by preganglionic cervical sympathetic stimulation. Fig. 5 shows the dose-inhibition curves of guanethidine and GQ-1, obtained in 4 cats respectively. Then 50 per cent blocking doses were estimated from the dose-inhibition curves and the ratio of ED50 of GQ-1 to that of guanethidine was calculated as follows: ED50 (GQ-1) = 1.41 mg./kg. / ED50 (guanethidine) = 0.89 mg./kg. GQ-1 was found to be approximately three-fifth as potent as guanethidine by this experimental procedure.

Effects of GQ-1 on the Blood Pressure of Cats

Intravenous injection of 1 to 5 mg./kg. of GQ-1 produced a fall in blood pressure in 13 cats anesthetized with urethane. No pressor response was observed by the intravenous injection of GQ-1 in doses of 1 to 5 mg./kg. in all cats studied. In most experiments the blood pressure returned to normal level within 5 to 10 minutes. Pressor responses to 5 μ g./kg. of norepinephrine were

slightly potentiated by intravenous injection of GQ-1 in doses of 1 to 5 mg./kg., whereas the pressor responses to 1 mg./kg. of tyramine were markedly diminished (Fig. 4).

The pressor responses caused by the bilateral carotid occlusion were reduced by the intravenous injection of 5 mg./kg. of GQ-1 in 4 cats. This effect lasted for more than 2 hours (Fig. 6).

The pressor responses elicited by faradization of the decentralized left greater splanchnic nerve were reduced following the intravenous injection of 5 mg./kg. of GQ-1 in 2 cats (Fig. 7).

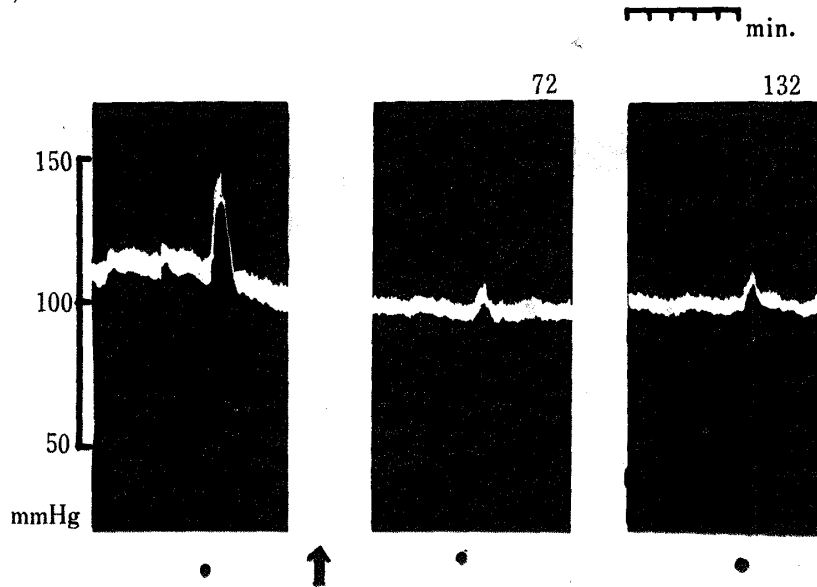


Fig. 6. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Pressor Responses elicited by Bilateral Occlusion of the Common Carotid Arteries in Urethane Anesthetized Cat

Occlusion of the carotid artery for 40 sec. at dots. At the arrow GQ-1 (5 mg./kg.) was given intravenously. Time notations refer to time elapsed after intravenous injection of GQ-1.

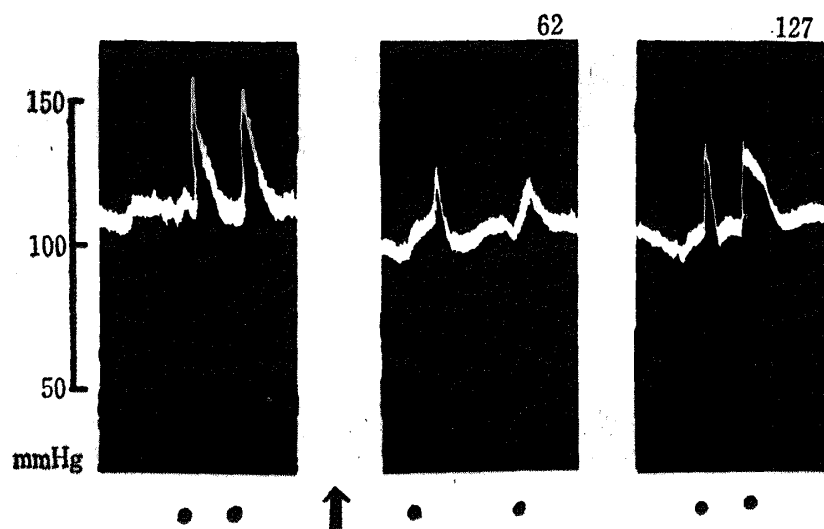


Fig. 7. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Pressor Responses elicited by Electrical Stimulation of the Greater Splanchnic Nerve in Urethane Anesthetized Cat

Stimulation of the splanchnic nerve (20 c.p.s., 0.7 msec. for 15 sec. at supramaximal voltage) at dots. At the arrow GQ-1 (5 mg./kg.) was given intravenously. Time notation refer to time elapsed after intravenous injection of GQ-1.

Effects of GQ-1 on Parasympathetic Efferent Transmission in a Dog and Cats

The depressor responses elicited by faradization of the cut vagal nerve were not affected following intravenous injection of 3 mg./kg. of GQ-1 in one dog and three cats. An example in dog is shown in Fig. 8.

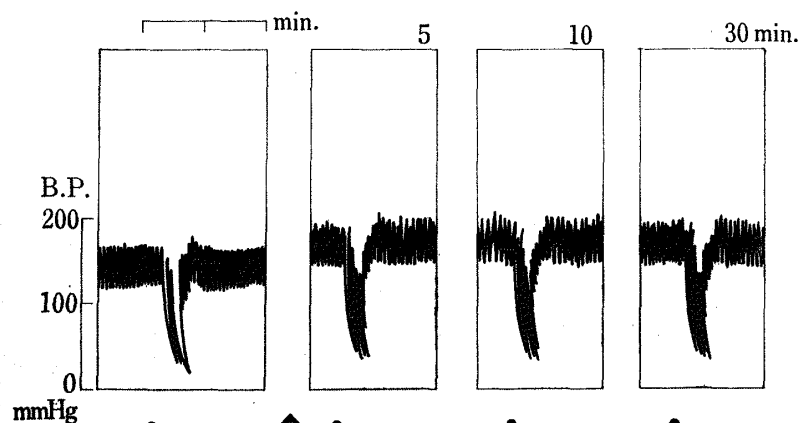


Fig. 8. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Depressor Responses elicited by Electrical Stimulation of the Vagus Nerve in Dog Anesthetized with Pentobarbital Na

Stimulation of the vagus nerve (20 c.p.s., 1 msec., for 15 sec. at 7 volt) at dots. At the arrow GQ-1 (3 mg./kg.) was given intravenously. Time notation refer to time elapsed after intravenously injection of GQ-1.

Effects of GQ-1 on the Isolated Rabbit Ileum

The inhibition of the pendular movements of the isolated ileum produced by the stimulation of the mesenteric nerve were abolished by GQ-1 in concentration of 1 μ g./ml.

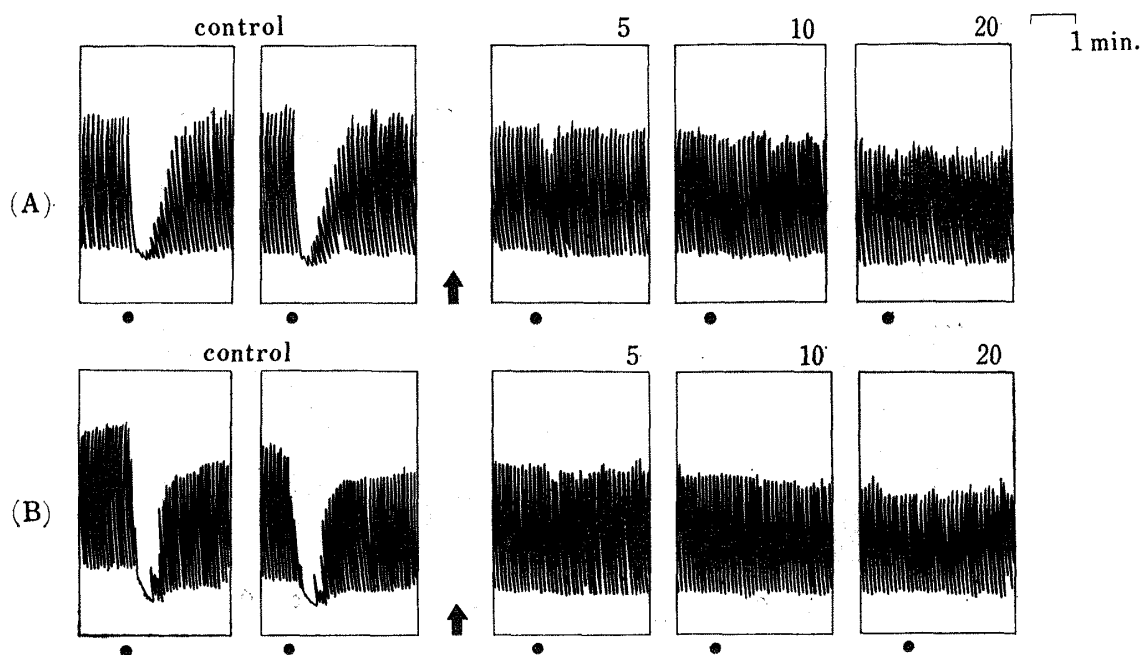


Fig. 9. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on Reduced Motility of Isolated Rabbit Ileum caused by Adrenergic Nerve Stimulation

Stimulation of the mesenteric nerve (20 c.p.s., 0.4 msec. for 20 sec. at supramaximal voltage) at dots. (A): at the arrow 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide sulfate (GQ-1) was added to the organ bath in a concentration of 1 μ g./ml.

(B): At the arrow bethanidine was added to the organ bath in a concentration of 1 μ g./ml. Time notation refer to time elapsed after administration of GQ-1 or bethanidine.

Similar results were obtained in the same concentration of bethanidine and guanethidine. An example is shown in Fig. 9.

Effects of GQ-1 on the Isolated Guinea-pig Heart

In the Langendorff preparations of the guinea-pig heart, the injection of 1 mg. of GQ-1 into the arterial cannula caused no change on the cardiac activities (Fig. 10).

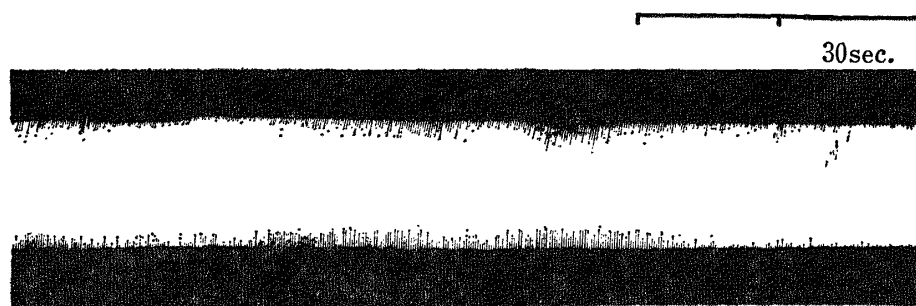


Fig. 10. Effects of 2,3,4,5-Tetrahydro-1*H*-2-benzazepine-2-carboxamide Sulfate(GQ-1) on the Isolated Guinea-pig Heart

At the dot injection of 1 mg. of GQ-1 was made into the perfusate.

Effects of GQ-1 on the Isolated Rat Diaphragm

Contractions of the isolated rat diaphragm in response to stimulation of the phrenic nerve were not affected by GQ-1 in a concentration of $10^{-4}M$.

Discussion

In the present study it was demonstrated that GQ-1 depressed the peripheral sympathetic nervous system, that is, GQ-1 inhibited the contractile response of the cat nictitating membrane to postganglionic sympathetic nerve stimulation, the pressor response of the cat blood pressure to the splanchnic nerve stimulation and the pressor response to bilateral carotid occlusion. The inhibiting effect of GQ-1 on response to preganglionic stimulation in the cat nictitating membrane was reversed by following administration of amphetamine. Action of GQ-1, in acute experiments on the cat nictitating membrane, depends upon the interference with sympathetic effector system through the action at the sympathetic nerve terminals. All of these effects on sympathetic function as mentioned above resemble to those of guanethidine,^{1,10,11)} and guanisoquin.⁴⁾ These results suggest that GQ-1 also depletes the catecholamines from peripheral nerve endings.

GQ-1 potentiated the pressor response of norepinephrine and reduced the pressor response of tyramine in cat. Potentiation of norepinephrine and the reduction of pressor response of tyramine, previously reported with guanethidine,¹⁾ indicated that GQ-1 also possessed the cocaine-like action. In this respect GQ-1 differs from guanisoquin, since the latter did not augment the response of norepinephrine.⁴⁾

Adrenergic neurone blocking activity of GQ-1 was compared with guanethidine and bethanidine. The results showed that the activity of GQ-1 was three-fifth as potent as that of guanethidine on urethane anesthetized cat nictitating membrane preparation and as potent as bethanidine on the isolated rabbit intestine.

In addition above mentioned results, GQ-1 showed a number of pharmacological properties which were not shown by guanethidine. GQ-1 did not cause the contraction

10) M.D. Day : Brit. J. Pharmacol., 18, 421 (1962).

11) R.A. Maxwell, A.I. Daniel, H. Sheppard, J.H. Zimmerman : J. Pharmacol. Exptl. Therap., 137, 31 (1962).

of the nictitating membrane, nor did the rise of the blood pressure in the cat. Furthermore it did not significantly increase the amplitude of isolated guinea-pig heart. These results indicated that GQ-1 lacked the initial sympathomimetic action of guanethidine and guanisoquin. This finding means that GQ-1 possess a weak amine releasing capacity or none. Moreover GQ-1 did not inhibit the depressor response elicited by the stimulation of the cut vagal nerve. This suggests that GQ-1 does not impair the parasympathetic nervous system.

From the results mentioned above, the mechanism of action of GQ-1 may be more similar to that of guanoxan¹²⁾ or Ph 881/7¹³⁾ rather than guanisoquin.

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12) M.J. Davey, H. Reinert : *Brit. J. Pharmacol.*, **24**, 29 (1965).

13) K. Hermann : *Acta pharmacol. et toxicol.*, **20**, 201 (1963).