Arch. Pharm. Res. 3(1), 23~30(1980)

Influence of Berberine on the Blood Pressure of Rabbits

Suk Tai Ko and Dong Yoon Lim

College of Pharmacy and College of Medicine, Chosun University, Kwang Joo 500, Korea

(Received 5 April 1980)

Abstract□Berberine, when administered into a ear-vein of the rabbit anesthetized with urethane, produced a long-lasting, dose-related fall in blood pressure, but intraventricular berberine did not elicit the hypotensive response. This hypotensive activity of berberine was not influenced by pretreatment of vagotomization and atropine. Depressor responses induced by berberine were not impaired by diphenhydramine, chlorisondamine, guanethidine and propranolol, but reduced significantly by phentolamine pretreatment. Berberine attenuated markedly pressor responses of norepinephrine and epinephrine. These results suggest that berberine causes the hypotensive activity that is attributable to alpha adrenoceptor blockade, but not to a direct relaxant effect upon vascular smooth muscle.

Keywords□Berberine—depressor activity—alpha adrenoceptor blockade

Berberine is a benzodioxoloquinolizine alkaloid that is isolated from *Coptis japonica* Makino, a member of the family *Ranunculaceae* and *Phellodenron amurense* Ruprecht, a member of the family *Rutaceae*. It also occurs in many other plants including the *Berberis* species (*Berberidaceae*) and the family *Menispermaceae*.[1,2]

This drug is clinically used in bacillary and amebic diarrhea, and diarrhea caused by dyspepsia and food poisoning as a antidiarrheal and antiprotozoal agent.[1]

In its structure, berberine shows resemblance to the benzylisoquinoline and aporphine alkaloids, thalicarpine, *d*-tetrandrine and papaverine.[3]

In many of its reported pharmacological actions, berberine exhibits a depressive action on excitable tissue, perhaps through inhibition of depolarization and repolarization, and this leads to hypotension; anticholinesterase action has been also reported.[4]

Creasy reported that berberine inhibited the biosyntheses of DNA, RNA, proteins and lipids, as well as the oxidation of glucose ^{14}C to $^{14}CO_2$ when incubated with S180 cells *in vitro*.[5]

Lim described that the methanol extract of *Phellodendron amurense*, a plant which contains berberine, appeared to possess the hypotensive activity in the cat and the rabbit, which may be due to dual mechanisms by interference with peripheral sympathetic function, α-adrenoceptor blocking action, and peripheral parasympathomimetic action, muscarinic action.[6]

In the present study, berberine was investigated in anesthetized normal rabbits to determine its mode of action *in vivo*, especially on the depressor action and to compare it with *Phellodendron amurense* in mode of hypo-

tensive activity.

EXPERIMENTAL

Experimental Animals

White mature rabbits of either sex, weighing 1.7~2.2kg, were anesthetized with urethane subcutaneously. The tracheal cannula was inserted to the rabbit which was then tied in supine position on fixing pannel. The body temperature was maintained at 37~38°C with a thermostatically controlled blanket and heating lamp.

Determination of Blood Pressure

The right common carotid artery was catherized with artery cannula and connected via an mercury manometer to a kymography for continuons monitoring of arterial pressure.

Each rabbit was left undisturbed for at least 30 minutes after completion of the operative procedures to permit cardiovascular parameters be to stabilized.

Administration of Drugs

All drugs were administered via a cannula inserted into ear vein, except intraventicular injection.

After the rabbit was positioned in a stereotaxic apparatus, polyethylene cannula (length 3cm, external diameter 1.5mm) was placed in the third left lateral ventricle, according to Moon's method.[7]

Drugs

The following drugs were used: berberine chloride (Seoul Pharmaceutical Co, Ltd), phentolamine mesylate (Ciba), guanethidine sulfate (Ciba). chlorisondamine chloride (Ciba), propranolol. HCl (ICI), diphenhydramine. HCl (J.P.), cyproheptadine. HCl (UEC & Co.), atropine sulfate (Merck), norepinehrine bitartrate (Sigma), epinephrine bitartrate (Sigma).

These drugs were prepared in 0.9% w/v sodium chloride solution on the day of experiment and stored in a refrigerator, except norepinphrine and epinephrine.

Norepinephrine and epinephrine were dissolved in 0.9% acidic saline (pH 4.0), respectively. Doses of all drugs were expressed as the base.

Statistical Analysis

Statistical significance between groups was determined utilizing the student's t-test. Data obtained from animals which served as their own control were analyzed for significance using t-test for paired observations. In addition, an analysis of variance was used where indicated. A P-value of ($P<0.05$) was considered to represent a significant change unless specifically noted in the text. Values given in the text refer to means with standard errors (S.E.).

RESULTS

Effect of Berberine on Arterial Pressure

Animals were allowed to stabilze for at least 30 minutes before experimental protocols were initiated. When cardiovascular parameters become stabilized, the intravenous administration of berberine in three groups of anesthetized rabbits at infusion rates of 0.5, 1.5 and 5.0 mg/kg, body weight for 20 second to one minute, resulted in dose-related decrements in blood pressure (Fig. 1, the left). The hypotensive response was rapid at the onset and was sustained at a relatively

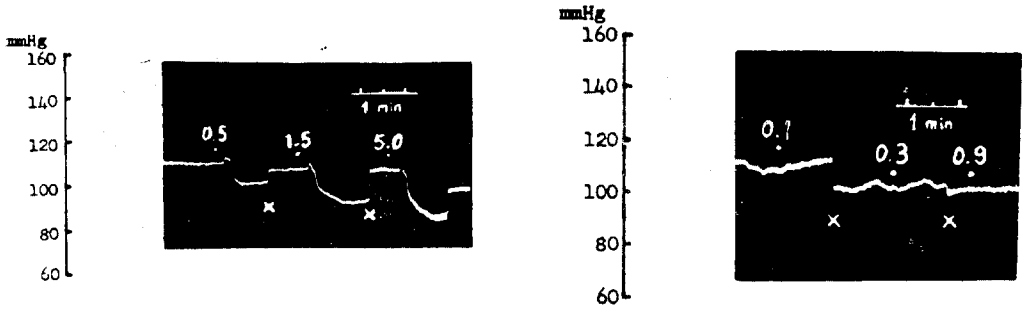

Fig. 1: Tracings of blood pressure of the whole rabbit to berberine.
Left: At the white dot, the indicated doses (0.5, 1.5 and 5.0mg/kg) were injected into a ear-vein.
Right: At the dot, berberine (0.1, 0.3 and 0.9mg/kg) were given into the lateral ventricle.
At X, tracing was stopped for 15~20 min. Each dose was injected at the intervals of about 15~20 min. Time: 1 min

Table I: Effects of berberine on the blood pressure of rabbits.

Administration route	Dose (mg/kg)	Changes of blood pressure (mmHg, fall from pre-injection level)	Number of animal
Intravenous injection	0.5	6.63±0.43	27
	1.5	12.96±0.76	27
	5.0	23.48±0.91	27

These results are average ± S. E. in mm-Hg.

constant level for 3~20 minutes. In the experiments of 27 rabbits, control blood pressure before administration of berberine was 95 ~120 mmHg, the magnitudes of hypotension were 6.63±0.43, 12.96±0.76 and 23.48± 0.91 mmHg in groups of rabbits receiving 0.5, 1.5 and 5.0 mg/kg i. v. of berberine, respectively, as shown in Table I.

In Figure 1, intraventricular administration of berberine was shown that any depsessor responses were not elicited at dosage of 0.1, 0.3 and 0.9 mg/kg of berberine.

Action of Atropine, Diphenhydramine, Chlorisondamine, Guanethidine and Propranolol on the Blood Pressure Effects of Berberine.

The influence of atropine, of diphenhydramine, of chlorsondamine, of guanethidine and of propranolol on the hypotensive action of berberine was studied in urethane-anesthetized rabbits. Each observation was reported in at least five to seven animals, respectively. The dosages of blockade of the specific receptors used in the present experiments were enough to block the effect of respective agonist (i.e., nicotine, acetylcholine, norepinephrine, isoprenaline and histamine).

The ganglionic blocking agent, cholrisondamine[8] (1.0 mg/kg) was slowly injected into an ear vein of rabbits. Depressor response induced by berberine was not affected by

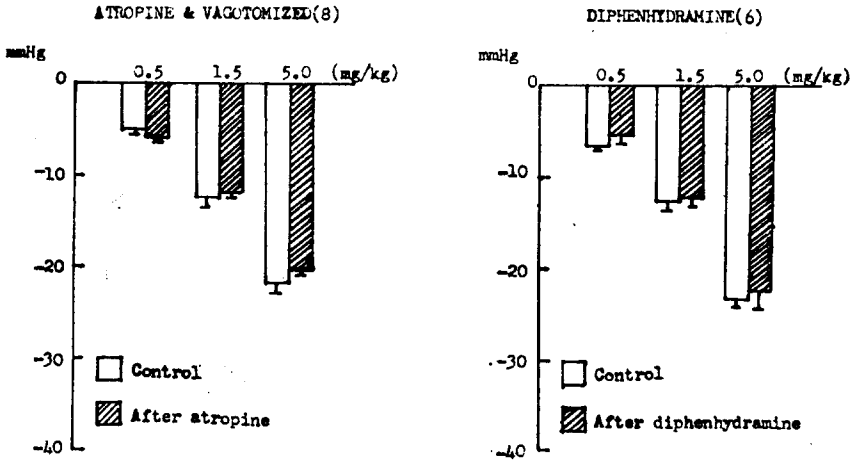

Fig. 2: Effects of atropine(left) and diphenhydramine(right) on the blood pressure of rabbits to berberine. Atropine(3mg/kg) and diphenhydramine(2mg/kg) were administered via a ear-vein immediately after control response in this experiment.
Ordinate: changes of blood pressure from preinjection level in mmHg.
Abscissa: dose of berberine. Numbers in bracket indicate numbers of experimental animal. Above all results are nonsignificant by comparing with control values.

chlorisondamine treatment.

This observation indicated that ganglionic transmission was not involved in the production of hypotension by berberine. Accordingly, a site of berberine action located more peripherally than the autonomic ganglia was considered (Fig. 3,the left). After inhibition of reponses to sympathetic adrenergic nerve action and to indirect acting sympathomimetic amines (e. g., tyramine, amphetamine) by gauanethidine[9] (10 mg/kg), the depressor action of berberine was not influenced (Fig.

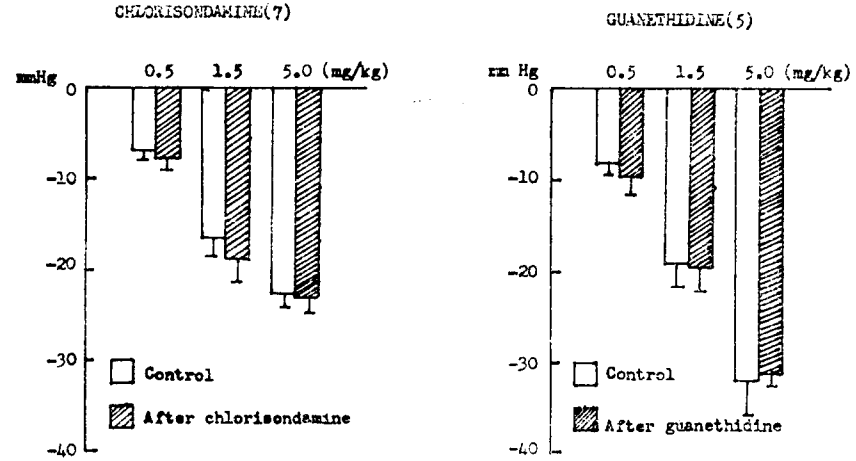

Fig. 3: Effects of chlorisondamine(left) and guanethidine(right) on the blood pressure of the rabbits to berberine. Chlorisondamine(1mg/kg) and guanethidine(10mg/kg) were administered intravenously in this experiment. Differences between the control value and value after each blockade are nonsignificant statistically. Other legends are the same as in Fig. 2

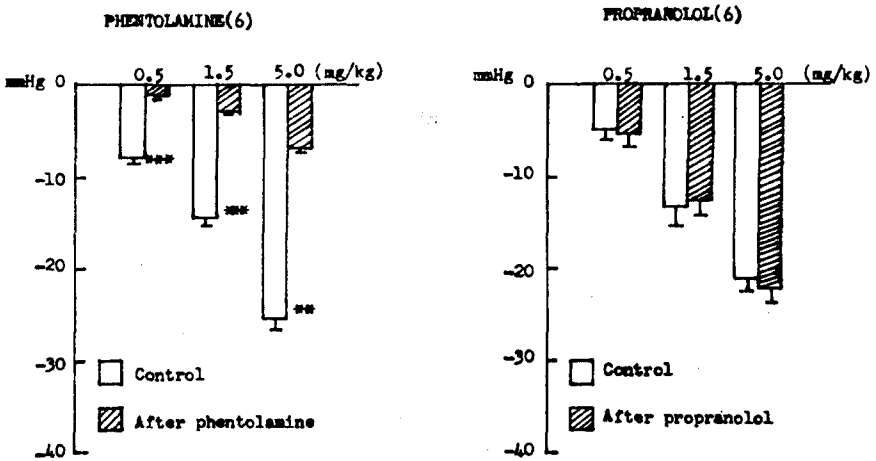

Fig. 4: Effects of phentolamine(left) and propranolol (right) on the blood pressure of rabbits to berberine.
Phentolamine(2mg/kg) and propranolol(2mg/kg) were injected intravenously. Other legends are the same as in fig. 2. ** $P<0.01$, *** $P<0.001$

3–right). Atropine (3.0 mg/kg) after bilateral vagotomization was given in order to blockade cholinergic receptor, but it did not affect the effect of berberine (Fig. 2 the left). Blockade of histamine receptors by a high dose (3 mg/kg) of diphenhydramine was also without effect on the action of berberine(Fig. 2, the right).

The effect of berberine on blood pressure before and after propranolol (2.0 mg/kg, i.v.) are shown in Fig. 4,the right. Propranolol, β–adrenoceptor blocking agent, did not alter the hypotensive response to berberine.

According to these observations, the hypotension elicited by berberine was not mediated by muscarinic receptors, histamine receptors or adrenergic neuron blocking action, nor was it due to a release by berberine of endogenous transmitter substances which affect cholinoceptors, β–adrenoceptors, muscarinic receptors, histamine receptors, or adrenergic nerve endings.

The Action of Berberine on the Blood Pressure Effects of Norepinephrine and Epinephrine in Anesthetized–Rabbits

In this series of experiments, a possible influence of berberine on the action of norepinephrine and epinephrine was studied. Each observation was repeated in five and six rabbits, respectively.

After control effects of the respective agents had been obtained, berberine was injected and the injections of the same agents were repeated during the hypotension elicited by berberine.

Figure 5 shows that effects of norepinephrine and epinephrine were distinctively inhibited during the hypotension induced by berberine. Norepinephrine (1.0 and 3.0 μg/kg, i. v.) given before starting the berberine injection produced pressor responses of 25.5 ± 5.67 and 46.67 ± 8.59 mmHg, respectively. Repetition of norepinephrine injection after berberine administration resulted in significantly reduced

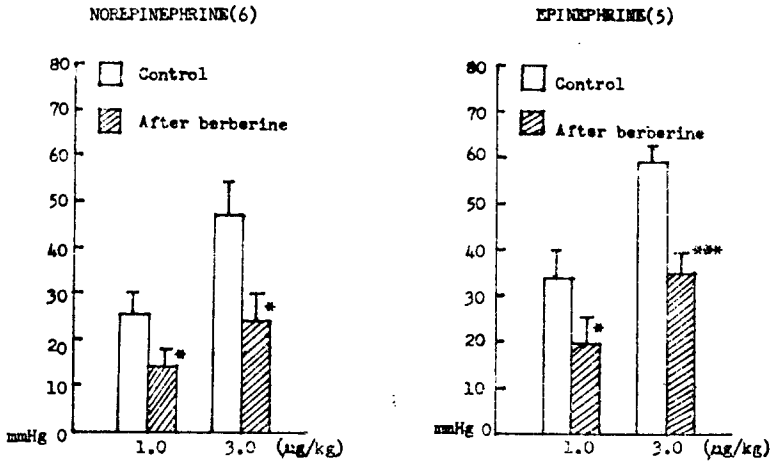

Fig. 5: Effects of berberine (5.0mg/kg) on the pressor action of norepinephrine(left) and epine-epinephrine(right) in the whole rabbits. Other legends are the same at Fig. 2.
* $P<0.05$, *** $P<0.001$

responses of 14.33±3.58 ($P<0.05$) and 24.33 ±6.72 ($P<0.02$) mm Hg, respectively.

Intravenous epinephrine (1.0 and 3.0 μg/kg, i. v.) was also decreased markedly from 33.6 ±5.23 and 59.2±2.75 mmHg of before berberine injection to 20.0±5.7 ($P<0.05$) and 34.6±4.62 ($P<0.001$) mmHg after berberine, respectively.

The Action of Phentolamine on the Hypotensive Effect of Berberine

Since berberine was found to inhibit the effect of norepinephrine and epinephrine on the rabbit blood pressure, a possible participation of blockade of α-adrenoceptors in the effect of berberine on the blood pressure was examined in more detail. Phentolamine (2.0 mg/kg) was injected intravenously in dose sufficiently high to ensure a complete blockade of α-adrenoceptors. A fall in the initial blood pressure followed the injection of phentolamine, and the effect of subsequently administered norepinephrine was abolished. In the present experiment, depressor responses of berberine were reduced. There was significant inhibition by phentolamine of the hypotensive effects of berberine in six rabbits.

Hypotensive responses of berberine (0.5, 1.5 and 5.0 mg/kg) given before the administration of phentolamine were 7.83±0.65, 14.50 ±1.20 and 26.83±1.11 mmHg, respectively, but after phentolamine markedly reduced responses of 1.17±0.31, 2.83±0.40 and 7.00 ±0.5 mmHg, respectively($P<0.001$), as shown in Fig. 3, the right.

DISCUSSION

Berberine was previously found to decrease mean blood pressure by Sabir *et al.*. In the present study, berberine injected into the ear vein of the rabbit in doses of 0.5, 1.5 and 5.0 mg/kg produced a marked, long-lasting, dose-dependent decrement in blood pressure, but intraventricular berberine did not induce the hypotensive action. Thus, the finding that berberine injected into the third ventricle did not elicit the hypotension suggests that the site of action of berberine is located

at the peripheral site.

The present experimental result shows that the hypotensive effect of berberine could not be inhibited by ganglionic blocking agent, chlorisondamine. This indicates that the action of berberine is exerted at a site distal to the autonomic ganglia. Further, the findings that the hypotensive activity of berberine was abolished by phentolamine, alpha adrenoceptor blockade, clealy show that berberine acts by interference with peripheral sympathetic function and that it is devoid of direct vasodilator activity. Among the drugs which interefere with peripheral sympathetic function, alpha adrenoceptor blocking agents alone cause reversal of the epinephrine pressor response.[9]

When epinephrine is administered to untreated animals, its alpha agonist properties predominate, resulting in a rise in mean arterial pressure. However, in the presence of alpha adrenoceptor blockade, the peripheral β_2-agonist properties of epinephrine predominate and a fall in arterial pressure or reversal of the pressor response is observed. In contrast the pressor responses to norepinephrine are impaired by alpha adrenoceptor blockade, but are not reversed,[10] as this agent possesses little β_2-agonist activity.[11]

In the present study, berberine significantly attenuated pressor responses to the alpha agonist, norepinephrime and the α-predominant agonist, epinephrine.

Thus, inhibition of berberine of the hypertensive effect of norepinephrine and epinephrine may be taken to present specific alpha adrenoceptor blockade.

These observations suggest that the hypotensive response of berberine is similar to that of prazocin, which is known as an antihypertensive agent, possessing alpha adrenoceptor blocking action,[12~14] but that its depressor activity is different with urotensin I on the mode of action *in vivo*.[15]

Urotensin is extracts of teleost fish urophyses which has a lowering property of blood pressure in rats.[16] It was reported that urotensin elicits the hypotensive action by directly dilatating the blood vessels.[16]

In view of the lack of any influence of atropine on the effect of berberine, it is difficult to propose a feasible interpretation of the inhibition by berberine of the blood pressure effect of acetylcholine in the rabbit. Therfore, cholinergic mechanism, muscarinic action, can be excluded, as the berberine effect is not antagonized by atropine pretreatment..

It may be of interest to note that this fact that it was not affected by atropine is disagreed with reports of Sabier *et al.*, in which berberine produces hypotension by anticholin esterase.[4] Furthermore, berberine does not act by inhibiting uptake of catecholamine in adrenergic nerve endings or by causing a release of histamine, an isoprenaline metabolites of adrenaline because hypotensive activity of berberine is not affected or changed by guan ethidine, diphenhydramine and propranolol.

Such a metabolite of adrenaline has been shown to be present in extracts of mammalian adrenal medulla,[17] in extracts of protein-free plasma of rabbits,[18] or released in a cat heart-lung preparation after stimulation of sympathetic chains.[19]

According to the above results, in the urethane-anesthetized rabbits, it appears that berberine produces a marked hypotensive activity by interference with peripheral sympathetic function (specific alpha adrenoceptor blockade), and that its depressor responsivity is similar to that of the extract of *Phellodenron amurense* as Lim reported in the previous studies.[6]

ACKNOWLEDGMENT

We thank Dr. Eun Hwa Lee of Seoul Pharmaceutical Co., Ltd., for supplying berberine.

LITERATURE CITED

1) Lee, S. J., and Lee, Y. J., *"Saeng Yak Hak,"* Seoul, Dong Myeong Sa, p. 63 and 99 (1971).
2) Lewis, W. H. and Elvin, M. P. F., *Medical Botany : Plants Affecting Man's Health,* John Wiley, New York (1977).
3) Creasey, W. A., in *Cancer : A Comprehensive Treaties* (F. F. Becker, Ed), Vol. 5, p.379, Plenum Press, New York(1977).
4) Sabir M. and Bhide, N. K. *Indian J. Physiol. Pharmac.* **14,** 111(1971).
5) Creasey, W.A., Biochemical effects of berberine. *Biochem. Pharmacol.* **28,** 1081(1979).
6) Lim. D. Y., Influence of *Phellodendri Cortex* methanol extract on the responses of the blood pressure in the rabbits and cats. *J. Korean Pharm. Sci.* **9,** 27(1979).
7) Moon. Y. B., Influence of intraventricular phenoxybenzamine on some actions of 5-hydroxytryptamine. *Chonnam Med. J.* **1,** 131(1964).
8) Goodman, L. S. and Gilman, A., *The Pharmacological Basis of Therapeutics,* 5th ed., Mac Millan Publishing Co., Inc., New York, p. 571, 553(1975).
9) Constantine, J. W., McShane, W. K., Scriabine, A., and Hess, H. J., Analysis of the hypotensive action of prazocin. In *Hypertension: Mechanisms and Management,* ed by G. Onesti, K. E. Kim and J. H. Moyer, Grume & Stratton Inc., New York, p.429(1973).
10) Freis, E. D., Mackey, J. C., and Oliver, W. F., The effect of "sympatholytic" drugs on the cardiovascular responses to epinephrine and norpeinephrine in man. *Circulation* **3,** 254(1951).
11) Ablad, B., Borg, K. O., Carlsson, E., Johnsson, G., Malmfors, I., and Regardh, C. G., A survey of the pharmacological properties of metoprolol in animals and man. *Acta Pharmacol. Toxicol.* **36,** *Suppl.* V, 7 (1975).
12) Cavero, I., Femard, S., Gomeni, R., Lefevre, F. and Roach, A., Studies on the mechanism of vasodilator effects of prazocin in dogs and rabbits. *European J. Pharmacol.* **49,** 100 (1978).
13) Graham, R. M., Oate, H. F., Stoker, L. M., and Stokes, G. S., α-Blocking action of the antihypertensive agent, prazocin. *J. Pharmacol. Exptl. Therap.* **201,** 747(1977).
14) Wood, A. J., Phelam, E. L., and Simpson, F. O., Cardiovascular effects of prazocin in normotensive and genetically hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **2,** 297(1975).
15) Lederis, K. and Medakovic, M., Pharmacological observations on the hypotensive action of extracts of teleost fish urophyses(urotensin I) in the rat. *Br. J. Pharmac.* **51,** 315(1974).
16) Kobayashi, H., Matsui, T., Hirano, T., Iwata, T. and Ishii, S., Vasodepressor substances in the fish urophysis. *Annot. Zool. Jap.* **41,** 154(1968).
17) Lockett, M. F., Identification of an isoprenaline-like substance in extracts of adrenal glands. *Br. J. Pharmac. Chemother.* **9,** 498(1954).
18) Roberts, D. J. and Lockett, M. F., A note on the influence of a metabolite of adrenaline on water diuresis in rats. *J. Pharm. Pharmac.* **13,** 631(1961).
19) Lockett, M. F., The transmitter released by stimulation of the bronchial sympathetic nerves of cats. *Br. J. Pharmac.* **12,** 86(1957).