injections, but dichloroisoproterenol blocks the effects of reserpine. It is concluded that reserpine acts directly on the gut, and that its effects are at least in part consequent to release of catecholamines from storage sites in the intestine.

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Influence of Vasopressors on Survival After Traumatic, Intestinal Ischemia and Endotoxin Shock in Rats.* (30190)

B. M. ALTURA, R. HSU, V. D. B. MAZZIA AND S. G. HERSHEY

Department of Anesthesiology, New York University Medical Center, New York City

PLV-2 (2-phenylalanine-8-lysine vasopressin) a synthetic vasoexcitor-pressor polypeptide, has been reported to increase survival of rats in hemorrhagic shock in which norepinephrine and angiotensin were ineffective(1, 2). Improved survival after PLV-2 administration was attributed to its selective vasomotor effects as observed in the microcirculation of the rat mesoappendix. Unlike norepinephrine which produced unselective intense constriction of all microvessels, or angiotensin which resulted in marked venular dilation and stasis, PLV-2 predisposed to improved true capillary inflow and outflow and reduced vascular sensitivity to epinephrine.

The use of vasoexcitor-pressor drugs in shock, as distinct from the hemodynamic principle of such therapy to improve tissue blood flow, is currently a matter of considerable uncertainty. It was of interest, therefore, to determine whether the favorable effects of PLV-2 in hemorrhagic shock also applied in other types of experimental shock. The present study compared the influence of norepinephrine, angiotensin and PLV-2† on survival of rats in traumatic, intestinal ischemia, and endotoxin shock.

Materials and methods. (A) Traumatic shock. Wistar strain female rats, 130-180 g body wt were fasted for 18-20 hours, except for water, and anesthetized with pentobarbital sodium, 2.0 mg/100 g body wt I.M. Normal saline, 1.0 ml/100 g was injected I.P. and one hour later the rats were subjected to 700 r at 40 rpm in a Noble-Collip drum. On removal from the drum a femoral vein was cannulated and 30 minutes post removal saline, 1.0 ml/100 g, alone or containing the various doses of the drugs‡ used (Table I) was infused I.V. over a 56-60-

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‡ Norepinephrine used in this study was Levophed® Bitartrate; 0.2% sol. = 0.1% base. All doses were calculated as the base. Angiotensin used was Hypertensin®. PLV-2 doses used were calculated on the basis of 20 μg of active polypeptide/I.U. (pressor).

minute period using a Harvard pump. The vein was ligated, incision closed and the animal observed for 48 hours for survival. Rats DOR or dying within 15 minutes after onset of infusion were not included in the data. All other animals were autopsied at death or sacrifice. Rats showing fractured skulls, subdural hematomata, lacerated viscera or torn vessels in the thoracic or abdominal cavities were also not included in the data. In selected experiments, arterial blood pressure (after removal from the drum), heart rate (ECG-lead II), serial hematocrit and rectal temperature were monitored for 2 hours after completion of the infusion.

(B) Intestinal ischemia (SMA) shock. Rats as in (A) were anesthetized with pentobarbital, 3.0 mg/100 g, and the superior mesenteric artery was temporarily ligated for 90 minutes using a previously described(3) technique. Effectiveness of ligation and resumption of blood flow were checked by noting acute onset of intestinal pallor and return of color accompanied by a fall and rise respectively in rectal temperature recorded by a thermistor (Y.S.I.) probe. The abdominal incision was temporarily closed during the period of ligation and permanently sutured after the above 90-minute period. A femoral vein was cannulated and 1 hour after removal of the arterial tie, saline, 2.0 ml, alone or containing the various drug doses (Table II) was administered as in (A). Survival was determined at 24 hours. All animals were autopsied. Rats dying within 15 minutes after the start of infusion were not included in the data. In selected experiments, arterial blood pressure, serial hematocrit and heart rate were monitored for 2 hours after completion of infusion.

(C) Endotoxin shock. Rats, 140-160 g, were anesthetized as in (B). Endotoxin,§ 2.0 mg/100 g from a freshly prepared isotonic glucose solution (20 mg/ml), was injected I.V. as a single dose. Four hours later saline, 1.0 ml, alone or containing the various drug doses (Table III) were infused as in (A). The 4-hour pre-treatment interval was selected because blood samples (tail vein) at this time, in all animals included in the series, showed increased total WBC with 40% or more neutrophils as well as hypocoagulability. Survival times were determined at 48 hours.

§ *S. enteritidis* #12047 (Difco Laboratories).

TABLE I. Influence of Vasopressor Drugs on Survival of Rats in Traumatic Shock.*

Drug†	Dose, μg/min	Survivors/Total	% Survivors‡
Normal saline	1.0 ml/100 g	19/56	34
Norepinephrine	.53	7/24	29
"	.03	9/26	35
Angiotensin	1.00	11/25	44
"	.10	15/33	45
PLV-2	.06	18/26	69§
"	.03	24/39	62§

* Noble-Collip drum—700 r (40 rpm).
† Drugs infused I.V. in saline over 50-60 min starting 30 min after drumming.
‡ Survival determined at 48 hr. All animals autopsied.
§ Statistically significant at 1% level (χ^2).

Results. (A) Traumatic shock. Survival rates (Table I) were significantly‖ higher at both dose levels in the PLV-2 treated group. Norepinephrine survivals were very similar to controls. The apparent trend toward increased survival in the angiotensin group was not significant. In all the drug treated groups initial blood pressure responses were prompt and their patterns generally resembled those previously reported after hemorrhage(2) during the course of drug infusion except that levels of systolic blood pressure reached with each drug were generally slightly lower (by 10-15 mm Hg). At the end of the infusion period pressures fell more rapidly and to lower levels in the norepinephrine group. There was no apparent correlation between eventual survival or death with blood pressure, hematocrit or temperature patterns in the selected experiments (8-10 animals) in each group except when drastic hypotension (30 mm Hg or lower) developed rapidly and death usually occurred during the course of the acute experiment.

(B) Intestinal ischemia (SMA) shock. Table II indicates that survival seemed to improve in all drug treated animals. However, the difference was significant‖ only in the PLV-2 groups. Here, too, other parameters monitored in some of the experiments

‖ Statistically significant at the 1% level (Chi-square).

TABLE II. Influence of Vasopressor Drugs on Survival of Rats in Intestinal Ischemia Shock.*

Drug†	Dose, μg/min	Survivors /Total	% Survivors‡
Normal saline	2.0 ml	3/45	7
Norepinephrine	.16	6/38	16
Angiotensin	.1	8/43	19
PLV-2	.03	28/46	61§

* Temporary ligation of superior mesenteric artery for 90 min.

†Drugs infused over 51-min period I.V. in saline starting 60 min after removal of arterial tie.

‡ Survival determined at 24 hr. All animals autopsied.

§ Statistically significant at 1% level (χ^2).

did not correlate with ultimate survival or death except when drastic hypotension developed rapidly during the acute experiment.

(C) Endotoxin shock. None of the drugs exerted a significant influence (Table III) on survival despite the apparent divergent trends from controls with both doses of angiotensin and with one of the doses each of PLV-2 and norepinephrine. The data on serial coagulation times, total and differential WBC, were not tabulated since, in this study, they served only as an index for the start of therapy. Their interrelationships with each other as a parameter of the progression of the syndrome is being investigated separately.

Discussion. While these preliminary data on survival in 3 types of experimental shock focus on the specific drugs used, they also have a direct bearing on the principle of vasoexcitor-pressor therapy in shock. Based on current information concerning the pathogenesis of shock, any therapy must be reconciled with its ultimate influence on tissue blood flow(4,5). The generally unfavorable experience with pressor drugs, based primarily on the use of amine pressors, does not, *per se*, negate the potentially favorable hemodynamic effects of the above principle for augmenting tissue flow. It can be inferred, therefore, that PLV-2 as contrasted with norepinephrine and angiotensin, predisposes to improved tissue blood flow in traumatic and SMA shock but not in endotoxin shock.

Circumstances which initiate shock can have their primary impact at any circulatory level on any of the factors determining tissue blood flow(6). Except for the final common pathway of microcirculatory insufficiency, the sequence of events which these initiating circumstances generate may differ qualitatively and quantitatively depending on the specific type of shock involved(7). Therapy also can influence tissue flow by modifying any of the numerous circulatory factors at any level. But its net effects, to be favorable, must permit of microcirculatory function adequate for essential oxidative metabolic needs(7).

In hemorrhagic shock it is generally agreed that microcirculatory failure results from spontaneous or induced restriction of splanchnic blood flow(4) when circulating blood volume is seriously reduced. There is much evidence(8,9) that procedures which improve microvascular mechanisms in hemorrhage are beneficial. The selective effects of PLV-2 on the terminal bed(2) account, at least in part, for its improvement of survival rates in hemorrhage. In SMA shock the sequence leading to peripheral insufficiency indicates that toxic materials released from ischemic intestine, in time, exhaust the capacity of the liver, particularly its RES component, to inactivate these materials which enter the general circulation(3,10) to affect microcirculatory smooth muscle mechanisms. Therapy designed to support hepatic function pharmacologically(10, 11) or by increasing its blood flow(12) is effective in SMA shock. Conceivably, PLV-2 may support liver function in SMA shock for a period sufficient to permit intrinsic compensatory mechanisms to cope with the materials released into the portal circulation. Such

TABLE III. Influence of Vasopressor Drugs on Survival of Rats in Endotoxin Shock.*

Drug†	Dose, μg/min	Survivors /Total	% Survivors‡
Normal saline	1.0 ml	9/25	36
Norepinephrine	.11	8/16	50
"	.024	6/20	30
Angiotensin	.11	2/14	14
"	.012	5/26	19
PLV-2	.11	5/14	36
"	.024	4/18	22

* *S. enteritidis* #12047 (Difco Labs), 2.0 mg/100 g body wt, I.V.

†Drugs infused over 51-min period I.V. in saline starting 4 hr after endotoxin injection.

‡ Survival determined at 48 hr. Results not significant at >5% level (χ^2).

effects could be due to the drug's selective actions on the microvessels of the liver if these are comparable with the findings in the mesentery and/or to its ability to increase the hepatic fraction of the cardiac output described by Berde(13) with the Rb^{86} technique in rats. In traumatic shock terminal vascular dysfunction is generated primarily by the same sequence as in hemorrhage in that a decreased circulating blood volume from local fluid loss(14) is a cardinal feature. In addition, in trauma, various tissue (biogenic) mediators and toxic materials have also been implicated. The latter presumably impair tissue function(15) because blood flow dependent detoxification mechanisms are depressed or inactivated. The mode of action of PLV-2 in trauma could include the pathways suggested for both hemorrhagic and SMA shock.

Endotoxemia in animals, like sepsis in humans, represents a special category in its pathogenesis compared with the other types of experimental shock discussed. The singular feature of endotoxemia is its early, major impact at the microcirculatory level to alter vascular myogenic mechanisms(16). Extreme hypersensitivity to catecholamines is succeeded by disruption of the responses of microvascular smooth muscle to the modulating effects of vasotropic substances. PLV-2, as administered here, is apparently not sufficiently effective in its attenuation of epinephrine reactivity or its selective effects on the microvessels to avoid insufficiency in the terminal bed. Yet experience with pressor therapy in septic shock is more favorable(17) than in other types of shock. But this same experience indicates that subjects are notably refractory to any therapy at that stage (as characterized by the blood picture) in which PLV-2 was used in this study. The time factor in therapy of endotoxin shock as related to parameters of its course requires further study.

The pathways whereby the drugs used do, or do not, affect survival in traumatic, SMA and endotoxin shock, in relating these drugs to their ultimate effects on tissue blood flow, are suggestive, being based on indirect evidence. However, the results directly support the principle of pharmacologic manipulation of the highly drug-sensitive microcirculatory mechanisms. This general concept of selective, local vasomotor actions of vasoexcitor substances is compatible with the intrinsic regulation of the microcirculation(18). It has also been demonstrated experimentally with exogenous vasoexcitor polypeptides(2,19,20, 21). In the absence of specific therapy in shock, drugs with selective vasoexcitor properties should be sought and their potential value explored.

Summary. The effects of norepinephrine, angiotensin and PLV-2 on survival of rats after traumatic, intestinal ischemia and endotoxin shock were compared. Epinephrine and angiotensin did not significantly influence survival in all 3 types of experimental shock. PLV-2 was significantly effective in traumatic and intestinal ischemia shock but not in endotoxemia. In the latter, the possible relationship of time of onset of drug therapy to the course of endotoxin shock was suggested.

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Antiviral Activity of Amantadine Hydrochloride in Tissue Culture and *in ovo*. (30191)

E. M. NEUMAYER, R. F. HAFF* AND C. E. HOFFMANN
(Introduced by J. A. Schneider)
Pharmaceutical Research Division, Industrial and Biochemicals Dept., E. I. du Pont de Nemours and Co., Wilmington, Del.

The antiviral activity of 1-adamantanamine hydrochloride† (amantadine HCl) in tissue culture, *in ovo* and *in vivo* has been briefly reviewed by Davies *et al*(1) and Maassab and Cochran(2). Demonstration of corresponding antiviral activity in human studies has been presented by Jackson *et al*(3) and Wendel(4). In this paper is presented a detailed account of the antiviral activity of amantadine HCl in tissue culture and *in ovo*.

Materials and methods. Compound. Solutions of amantadine were made either from the free base (neutralized with HCl) or from the hydrochloride salt.

Media. All media contained 100 units of penicillin G and 100 μg of streptomycin sulfate. The basic maintenance medium consisted of 90% Earle's saline(5) modified to contain 0.9% NaCl, 0.45% glucose, 0.015% phenol red and 0.5% enzymatic lactalbumin hydrolysate (Nutritional Biochemicals) and 10% Difco tryptose phosphate broth with 0.1% yeast extract and 290 mg% glutamine added. For studies on plaque formation 0.9% agar and for hemadsorption studies 0.3% agar was added to the liquid maintenance medium. Growth medium consisted of 2% normal chicken serum, 8% horse serum, 10% tryptose phosphate broth and 80% modified Earle's saline plus an additional 0.31% $NaHCO_3$.

Cells. Chick embryo cells were prepared from decapitated whole chick embryos according to the method of Dulbecco(6) and 25×10^6 cells in 10 ml of growth medium were placed in petri dishes. After a 48-hour incubation period the cell layers were washed with phosphate-buffered saline (PBS), inoculated with test virus and returned to the incubator for a one-hour virus adsorption period. Unadsorbed virus was then removed and the cell layers covered with 10 ml of maintenance medium and reincubated. All tissue cultures were incubated at 37°C in a humidified atmosphere (RH 90-95%) containing 10% CO_2. Other cell layers were treated in a similar manner. Plaque formation was established after an appropriate incubation time, usually 3 to 5 days, by addition of either 0.007% neutral red or 0.15% of 2(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT). Hemadsorption tests were developed by washing the infected monolayers with PBS after removal of the soft agar overlay and adding 3 ml of a 0.5% suspension of chicken red blood cells (RBC) in PBS. Excess RBC were washed off after 20 minutes and the monolayers checked for areas of adsorbed RBC. Tests for total virus were carried out with liquid maintenance medium. At the end of the desired incubation period the infected cells were scraped into the medium and subjected to 3 cycles of freezing at —70°C and thawing at 37°C. The cell debris was removed by centrifuga-

* Present address: Smith Kline & French Laboratories, Philadelphia, Pa.

† Symmetrel, E. I. du Pont de Nemours and Co.