Effect of Diazoxide on Vascular Responses to Norepinephrine in the Calf of Normal Subjects.* (28147)

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Chlorothiazide, a benzothiadiazine diuretic and antihypertensive agent, reduces the vasoconstrictor effect of norepinephrine in normal subjects(1). Diazoxide (7-chloro-3-methyl-1,2, 4-benzothiadiazine-1, 1-dioxide), a nondiuretic benzothiadiazine which has been synthesized recently, also has antihypertensive properties(2-6), but its effect on the vasoconstrictor responses to norepinephrine is unknown. The present studies were undertaken to see whether it reduces the vascular responses of normal subjects to norepinephrine.

Materials and methods. The experiments were carried out on 6 healthy young men. They were studied while lying in the supine position. Room temperature was maintained at 82-84°F. A needle was placed in a superficial vein of the right arm and connected by means of a 3-way stopcock to a bottle containing a solution of norepinephrine in 5% glucose in water and to a motor-driven syringe. The syringe was used to deliver norepinephrine bitartrate at constant rates of 0.0375, 0.075 or 0.15 μg of norepinephrine base/kg/min.

The right leg was placed in a limb-segment plethysmograph and suspended from the heel and lower thigh by slings padded with foam rubber. The posterior aspect of the leg was at heart level. Care was taken to have the leg in a comfortable position and to avoid venous compression by the upper sling. A loosely fitting sleeve of thin neoprene rubber enclosed the limb. The open ends of the sleeve were everted and permanently attached to flanges at the ends of the plethysmograph. The spaces between the limb and the margins of the orifices at each end were closed by 6 wedge-shaped metal plates adjusted to fit the contour of the limb and held in place by wing nuts. The water in the plethysmograph forced the neoprene sleeve into loose folds against the skin and inner surfaces of the metal plates. The water temperature within the plethysmograph was maintained at 90°F. The water level in the instrument was adjusted so that its pressure on the leg was slightly greater than venous pressure. This reduced transmural venous pressure to a low constant value(7) so that the veins could be distended freely when venous congestion was produced by suddenly inflating a pneumatic cuff on the right thigh just above the knee. The proximal end of the plethysmograph was just distal to the patella. A cuff about the leg distal to the plethysmograph was inflated to suprasystolic pressure during blood flow observations to exclude the circulation in the lower leg and foot. Changes in limb volume were measured as changes in the height of a column of water in an open tube attached to the top of the plethysmograph. The change in water level was sensed as a change in pressure using a Sanborn pressure transducer. Heart rate was obtained from a pulse tracing recorded from a microphone taped over a digital artery. Arterial pressure was measured by auscultation with a cuff on the left arm. Room temperature and the temperature of the left great toe were monitored with a thermistor and used as a guide to equilibration of the cardiovascular system with the environment. The digital pulse and the volume changes in the plethysmograph were recorded with a Sanborn direct-writing oscillograph.

Blood flow was calculated from the rate of increase in leg volume with venous occlusion and expressed in ml/100 ml of leg volume/min. Control values represent averages of observations made after flow, skin temperature, blood pressure and heart rate became stable before the first infusion of norepinephrine,

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TABLE I. Parameters of Regression Equations for Each Session.

Variable	Session	b	Limits		$\bar{y}$	Limits	
Pulse rate	A	−107.19	− 25.26	−189.12	57.71	62.24	53.18
	B	− 92.27	− 22.54	−162.00	56.58	60.44	52.72
Mean blood pressure	A	194.03	279.27	108.79	113.71	118.43	108.45
	B	198.10	285.21	110.99	111.73	116.56	106.90
Blood flow	A	− 5.02	− 2.17	− 7.87	1.75	1.91	1.59
	B	− 4.48	− .45	− 8.51	2.16	2.38	1.94
Vascular resistance	A	295.79	419.72	171.86	71.08	77.91	64.25
	B	211.67	290.29	133.05	52.26	58.33	54.19

The limits include upper and lower values for 95% confidence intervals.
Regression equations were computed using the actual values for doses of norepinephrine.
Regression equation is: $Y - \bar{y} = b\,(X - \bar{x})$.

Experimental values are averages of all observations made between the beginning of the second and the end of the sixth minute of infusion. Mean blood pressure was computed as the diastolic plus one-third of the pulse pressure. Resistance, expressed in arbitrary units, was obtained by dividing mean pressure by the corresponding blood flow.

Subjects rested for at least 30 minutes before the experiment was started. Observations on blood flow, blood pressure, pulse rate and temperature were made intermittently after this rest period until they became stable. Norepinephrine was then infused for 6 minutes. Blood flow was recorded at 20-second intervals and blood pressure was taken at 30-second intervals during the infusions. The order of infusion for each subject was determined from a table of random numbers(8). The subject was allowed to rest for 24 minutes between infusions. During this time blood pressure, heart rate and skin temperature returned to control levels. Blood flows were recorded for 4 minutes just before the second and third infusions to be sure the control levels were reached. The procedure was carried out twice in each subject. The first session (A) served as the control. The second session (B) was held 1 week after A. During this interval each subject received 300 mg of diazoxide daily, but performed his usual activities without dietary restrictions. Subjects reported to the laboratory at the same hour each day at which time they were weighed and given the diazoxide. At the beginning of each session 15 ml of venous blood was taken for measurement of the hematocrit and the serum sodium, potassium and chloride.

Dose-response regressions were computed (9) for pulse rate, blood pressure, blood flow and resistance. The reported values for "zero dose" are the averages of observations made during the final 4 minutes of the control period preceding the first norepinephrine infusion. Reported values for each of the 3 doses are averages of all the observations made from the beginning of the third to the end of the sixth minute of infusion.

Parallel line bioassays were carried out whenever the regressions of responses on the logarithm of the norepinephrine dose for both sessions were linear and parallel and satisfied the other requirements for such assays(10). These assays were done by treating norepinephrine in session A as the standard preparation and norepinephrine in session B as the test preparation. Since the doses of norepinephrine were the same in both sessions, the calculated potency ratio and its fiducial limits served as a statistical expression showing a difference between responses of subjects to norepinephrine before and after treatment with diazoxide. Whenever the regressions did not meet the requirements for a parallel line assay, the responses in the 2 sessions were compared by considering the mean values and the confidence limits for the responses to all doses in each session.

Results. Dose-response curves were computed for pulse rate, mean blood pressure, blood flow and resistance. The slopes of the regression equations and the mean values for the responses and their 95% confidence limits are in Table I.

Mean blood pressure increased and heart rate decreased linearly with the dose of norepinephrine in both sessions. The absolute

TABLE II. Weight of Subjects (in kg) During Treatment with Diazoxide.

Subjects	Days 1	2	3	4	5	6	7	8
M.G.	78.4	78.9	78.7	79.3	77.8	79.3	79.1	79.4
D.K.	71.1	71.7	71.8	72.6	72.3	71.9	72.6	72.3
R.C.	81.1	81.3	81.3	82.4	82.3	82.8	82.3	82.1
M.W.	85.7	86.0	85.9	86.6	86.7	86.6	86.6	86.1
J.McD.	78.6	79.9	80.0	79.4	80.1	80.8	80.8	80.8
J.N.	92.3	93.5	93.2	93.2	93.1	94.3	94.7	92.5
Avg	81.2	81.9	81.8	82.3	82.1	82.6	82.7	82.2

The subjects received 300 mg/day of diazoxide from day 1 to day 7. Sessions A and B were held on 1st and 8th day respectively.

levels achieved at each dose were not altered significantly by the diazoxide. Calf blood flow was significantly higher with norepinephrine after diazoxide and vascular resistance was significantly lower (Table I).

Vascular resistance, mean blood pressure and pulse rate were compared by means of the bioassay. The assay revealed that the average dose of norepinephrine necessary to produce the same level of resistance in session B was 3.03 times greater than the dose required in session A. The doses of norepinephrine required to produce a given blood pressure or heart rate were the same in both sessions. Blood flows could not be compared by the same technic of assay because the responses were not linearly related to the logarithm of the norepinephrine dose.

Table II shows that body weight tended to increase progressively during the week of observation. There was a very slight fall in serum sodium in each subject and a slight fall in the hematocrit. There was no apparent effect on serum potassium or chloride (Table III).

TABLE III. Serum Electrolytes Before and After Diazoxide Therapy.

Subjects	Session	Na, meq/l	K, meq/l	Cl_2, meq/l	Hct, %
M.G.	A	140.8	4.1	103.8	43.19
	B	140.6	4.1	107.9	42.80
D.K.	A	142.2	4.6	108.9	44.00
	B	141.4	4.8	108.1	40.44
R.C.	A	142.0	4.0	111.6	43.81
	B	141.5	4.0	110.6	42.67
M.W.	A	143.5	4.3	105.5	41.74
	B	142.2	4.3	104.3	39.05
J.McD.	A	142.5	3.9	107.6	42.44
	B	141.5	4.0	103.6	39.56
J.N.	A	143.5	4.3	106.1	46.42
	B	143.0	4.3	106.3	43.86
Avg	A	142.4	4.2	107.3	43.60
	B	141.7	4.3	106.8	41.39

A represents control session and B represents session held 1 week after A.

Summary. In these experiments the administration of diazoxide, 300 mg per day for 7 days, had no significant effect on blood pressure or heart rate responses to norepinephrine. Blood flow at each dose level of norepinephrine was significantly higher after diazoxide and vascular resistance was significantly lower. The lower vascular resistance at the same level of transmural pressure indicates that the tone present in the vessel walls at each dose of norepinephrine was less after diazoxide. Body weight increased progressively during the week of treatment and there was a slight decrease in hematocrit. There appeared to be no major changes in the serum electrolytes. In these experiments diazoxide, a non-diuretic compound, reduced the vascular responses to norepinephrine. In this respect it resembles chlorothiazide, its diuretic congener.

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Immunological Neutralization of Various Erythropoietins.* (28148)

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Serum from rabbits immunized with an extract of erythropoietically active human urine will neutralize *in vitro* the erythropoietic activity of such extracts. Also, injections of such immune serum into normal mice will depress erythropoiesis, indicating that endogenous erythropoietin of mice can also be neutralized and that normal erythropoiesis in mice is erythropoietin dependent. Both neutralizations are considered to be the result of an antigen-antibody reaction directed against either erythropoietin or the general class of molecules to which erythropoietin belongs (1,2).

Erythropoietically active material has been found in plasma and in urine of several animal species. Some of these erythropoietins have been effective in stimulating erythropoiesis when injected into animals which differ in species from the source of the administered erythropoietin(3). The purpose of the present investigation is to study, using immunological methods, the interspecies relationships of erythropoietin.

Materials and methods. An extract of the urine of a patient with aplastic anemia was prepared by ultrafiltration through a collodion membrane. This extract had erythropoietic activity equivalent to approximately 20 cobalt units per milligram dry weight. An alum precipitate of the urinary extract was prepared by the method of Kabat and Mayer (4). Rabbits were immunized with a suspension of the alum precipitated urinary erythropoietin containing 1.5 mg of extract per ml. The rabbits were immunized by intravenous injection 3 times a week, following an initial injection given intraperitoneally. The volume of the alum precipitated suspension injected varied as follows: 3 injections of 1 ml; 3 injections of 1.5 ml; 4 injections of 2 ml; 4 injections of 3 ml; and 2 injections of 5 ml. The animals were bled on the fifth to seventh day after final injection and the serum collected. The ability of such serum to neutralize erythropoietin was determined by its effect in depressing the 24-hour red blood cell incorporation of Fe^{59} in normal mice after 4 daily subcutaneous injections of 0.25 ml of serum per day. The immune serum used in this study depressed the Fe^{59} incorporation to 1.5% from a value of 26.9% for mice similarly treated with normal rabbit serum.

An hypoxic environment or production of a severe anemia by injection of phenylhydrazine was used to elevate the serum erythropoietin levels. Groups of rats and rabbits and a single sheep were exposed in a decompression chamber to an oxygen tension equivalent to that of 20,000 ft altitude (approximately $pO_2 = 72$ mm Hg). All animals were bled within 30 minutes after 24 hours of hypoxic exposure; the sheep was bled again after 48 hours of exposure. Anemia was produced with phenylhydrazine in rabbits and mice. Rabbits were injected daily for 6 days with a solution of 2.5% phenylhydrazine hydrochloride, 2 ml on the first day and 1 ml on each subsequent day, and then bled the day following the last injection. Mice were given 4 injections of 1.5 mg phenylhydrazine hydrochloride per injection over a 6-day period and bled on the seventh day. Mice with transfusion-induced polycythemia were used for erythropoietin assay using a procedure slightly

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