Cardiovascular Effects of Su-4885 (Mepyrapone), An Inhibitor of Corticosteroid Biosynthesis.* (28912)

ALLAN M. LEFER AND GEORGE R. NADZAM (Introduced by R. M. Berne)
Department of Physiology, University of Illinois, Urbana and Department of Physiology, Western Reserve University, School of Medicine, Cleveland, Ohio

Su-4885 [mepyrapone or 2-methyl-1,2-bis-(3-pyridyl)-1-propanone] inhibits the biosynthesis of physiologically important corticosteroids(1,2). In the dog, intravenous administration of 75 mg/kg of Su-4885 results in a marked reduction in the secretion of cortisol and of corticosterone by the adrenal (3). Onset of the reduction occurs within 15 minutes after injection of Su-4885 and persists for about 7 hours. Chart *et al*(1) reported that high oral doses of Su-4885 caused motor ataxia and prolific salivation in dogs. However, even at doses of 200 mg/kg, there were no fatalities(2).

Stone(4) reported that Su-4885 does not alter the cardiovascular response to epinephrine in intact dogs. Lefer(5) has shown that oral administration of Su-4885 to intact dogs does not appreciably alter cardiovascular function measured 2 to 4 hours later. However, since intestinal absorption of Su-4885 may be irregular or slow, and since a rapidly occurring effect may go undetected, intravenous infusions of Su-4885 were undertaken. Data are presented here which demonstrate that Su-4885, given intravenously, in concentrations less than or equal to those which inhibit corticosteroid biosynthesis has a direct depressant action on the cardiovascular system.

Methods. Intact dogs. Fifteen male mongrel dogs (16.3 to 21.2 kg) were fasted 24 hours prior to use. Su-4885 bitartrate† was infused intravenously *via* a femoral vein at rates of from 1 to 6 mg/kg/min (in a volume of 2 ml/min). In control experiments, corresponding amounts of tartaric acid were infused at the same rates.

All observations were made in closed-chest dogs respiring spontaneously. A Grass model 5 Polygraph was used to record left ventricular contractile force (LVCF), mean femoral arterial blood pressure (MABP), aortic flow and the electrocardiogram. Cardiac contractile force was measured with a modified strain gauge arch(6) sutured to the epicardial surface of the left ventricle. The segment of myocardium just under the gauge was stretched about 25 to 35% of its diastolic length. Arterial blood pressure was measured by a Statham pressure transducer model P23-AC (frequently calibrated against a mercury manometer), aortic flow, by a gated sine-wave electromagnetic flowmeter. The flow probe was positioned around the ascending aorta proximal to the origin of the brachiocephalic artery. The heart and great vessels were exposed by a thoracotomy in the left fourth intercostal space. A branch of the right femoral artery and of the vein were cannulated for measurement of arterial blood pressure and for infusion of the Su-4885, respectively.

After the chest was sealed, positive-pressure respiration was discontinued and a 45- to 60-minute equilibration period elapsed before the Su-4885 was administered.

Isolated cat papillary muscle. The methods used for the cat papillary muscle preparation were those employed by Tanz and Kerby(7) with certain modifications. Cats of either sex were anesthetized with ether; cardiectomy was rapidly performed and the papillary muscles were excised from the right

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† Su-4885 in the form of the free base was generously donated by Dr. J. J. Chart of Ciba Pharmaceutical Co., Summit, N. J. The bitartrate was prepared by adding one mole of Su-4885 (M.W. 226) to 2 moles of tartaric acid (M.W. 150) and bringing up to volume in 0.9% sodium chloride solution. For example, if one were to administer 50 mg/kg of Su-4885 bitartrate to a 20 kg dog, then 1.0 g of the free base would be added to 1.333 g of tartaric acid, and the mixture brought up to 20 ml with 0.9% sodium chloride solution.

TABLE I. Effects of Intravenous Su-4885 Infusions on Cardiovascular Performance.

Parameter	Infusion rate 1 mg/kg/min	2 mg/kg/min	4 mg/kg/min	6 mg/kg/min
LVCF* (10 exp)	89 ± 1.1	70 ± 1.4	57 ± 1.8	48 ± 2.2
MABP* (10 exp)	93 ± 1.8	75 ± 2.1	58 ± 2.3	52 ± 3.1
Heart rate* (10 exp)	94 ± 2.8	87 ± 2.6	82 ± 3.0	74 ± 3.2
Aortic flow* (4 exp)	84 ± 5.4	69 ± 5.5	55 ± 4.6	47 ± 4.5
Recovery time (min) (10 exp)	1.5 ± .2	9.4 ± 1.5	18.0 ± 2.1	34.2 ± 5.5

* Values are end-infusion values expressed as % of pre-infusion control level ± S.E.

ventricle with their chordae tendinae intact. The papillary muscles were immediately placed in a 15 ml bath containing Krebs-Henseleit solution. A mixture of 95% O_2 and 5% CO_2 was bubbled through the solution maintained at a temperature of 37.5 ± 0.2°C. The papillary muscles were electrically driven by a pulse generated by a Grass model S-4 stimulator directly coupled to field electrodes in the bath. A stimulus of 20 msec in duration, two volts above threshold (which was found to be about 0.5 to 2.0 volts) was applied at a frequency of one per second. Contractile force was continuously monitored by Grass Force Displacement transducers (model FT-03) and recorded by a Grass model 5 Polygraph. The muscles maintained uniform contractile forces for several hours at initial tensions of one to two grams.

Dose-response relations of Su-4885 were determined on 12 papillary muscles. The volume of solution added to the bath varied from 0.1 to 1.0 ml. Equivalent volumes of Krebs-Henseleit solution exerted no detectable inotropic effect. The Su-4885 was washed out and fresh Krebs-Henseleit solution (maintained at 37.5°C and aerated with 95% O_2 and 5% CO_2) was added after each dose of Su-4885. The muscle returned to its control contractile force after a short period of recovery and overshoot.

Significance of results was evaluated by use of appropriate "t" tests; the level of significance is indicated by P-values.

Results. 1. *Intact dogs.* The effects of intravenous infusion of various doses of Su-4885 bitartrate on cardiovascular performance are shown in Table I. The observations were made at the end of infusion of the drug (end-infusion values) and represented the maximal level of inhibition obtained. Recovery times (period from termination of infusion until all variables return to pre-infusion control levels) are also given in Table I. After recovery the next dose was administered. In most experiments, increasingly higher doses were given; the sequence was altered in a few experiments (highest dose first) without any noticeable change in response to the drug. Infusions of corresponding amounts of tartaric acid in 0.9% sodium chloride solution had no detectable effect on any of the variables monitored.

From Table I it is evident that Su-4885 decreases LVCF, MABP, heart rate and aortic flow in proportion to the dose employed. Furthermore, recovery times were dose dependent. At the highest rate of infusion reported (*i.e.*, 6 mg/kg/min), cardiac failure and death resulted in 3 cases. This failure was characterized by a rapid and progressive decrease in LVCF, MABP, aortic flow and heart rate, accompanied by arrhythmias. Infusion of 8 mg/kg/min of Su-4885 resulted in a fatality in 5 out of 6 dogs.

In 2 dogs, cortisol succinate (5 or 8 mg) was added to the regular infusate of Su-4885 bitartrate. In both animals, the changes were similar to those obtained in the experiments in which Su-4885 was infused alone. It would appear that Su-4885 exerts its cardiovascular depressant effect in presence of high corticosteroid concentrations. The actions of Su-4885 on the cardiovascular system are independent of the concentrations of corticosteroids in the blood.

2. *Cat papillary muscles.* Table II summarizes results obtained in isolated cardiac tissue. Contractile force progressively diminished as concentration of Su-4885 was increased. The difference between any 2 responses is statistically significant ($P<0.001$).

TABLE II. Inotropic Action of Su-4885 on Isolated Cat Papillary Muscle Preparation.

Conc of Su-4885 in bath (mg/ml)	Contractile force (% of control)	Time to peak response (sec)	Recovery time after washout (sec)
.022 (12)*	84.2 ± 1.1†	143.7 ± 7.8†	2.14 ± .17†
.044 (12)	72.0 ± 1.3	137.5 ± 8.2	3.44 ± .28
.111 (16)	51.0 ± 1.5	146.5 ± 8.1	9.20 ± 1.43
.178 (9)	36.9 ± 1.0	160.0 ± 9.7	12.90 ± 1.48
.222 (12)	27.7 ± 1.0	149.2 ±12.1	17.90 ± 1.93

* No. in parentheses indicates total No. of Su-4885 administrations at a particular dose level; a total of 12 papillary muscles were used.
† ± S.E.

However, the duration of time required to attain the maximal negative inotropic effect (time to peak response) was not dose dependent.

Recovery time, defined as time between addition of fresh Krebs-Henseleit solution and restoration of pre-Su-4885 contractile force, closely paralleled the dose employed ($P<0.01$ for difference between any 2 recovery times).

Discussion. Su-4885 has been shown to exert a depressant action on the cardiovascular system of the intact dog and on the isolated cardiac muscle of the cat, at concentrations of 10^{-4} to 10^{-5} M. In the whole animal and in isolated cardiac tissue there is a definite dose response relationship. The cardiovascular actions of Su-4885 in the whole animal may be explained on the basis of inhibition of myocardial contractility or inhibition of vascular smooth muscle. The fact that a similar degree of depression of cardiac contractile force was observed in the cat papillary muscle preparation suggests that at least one action of Su-4885 is a direct one on cardiac muscle to inhibit contractile force which in turn explains the decrease in arterial blood pressure and in blood flow found in the dog. The possibility cannot be excluded that the drug also acts on the peripheral vasculature to reduce resistance and produce venous pooling of blood.

The cardio-inhibition induced by Su-4885 in the dog is a temporary and reversible phenomenon. This may indicate that the affinity of the drug for its receptor site in or on cardiac muscle cells is weak. The fact that a single washout of the buffer medium in the bath of the cat papillary muscle preparation was sufficient to restore contractile force to control levels in a matter of a few seconds supports this idea. However, since the biological fate of the compound (routes of inactivation and excretion) are unknown, one cannot rule out metabolic factors in the rapid recovery in the whole animal.

If one assumes that Su-4885 is distributed in the extracellular fluid compartment (20% of body weight taken as an estimate of extracellular fluid volume), then there is a striking similarity between the effective concentration range of Su-4885 in the cat papillary muscle preparation (0.02 to 0.22 mg/ml) and that in the intact dog (0.04 to 0.24 mg/ml).

One might be tempted to ascribe the depression of cardiovascular function induced by Su-4885 to corticosteroid insufficiency. However, since Su-4885 inhibited the cardiovascular system in the presence of high concentrations of cortisol the actions of this drug appear to be corticosteroid independent.

Summary. Su-4885 decreased myocardial contractility, arterial blood pressure, aortic blood flow and heart rate in intact dogs. Degree of cardiovascular inhibition as well as recovery time were dose dependent. The drug inhibited cardiovascular function in the presence of relatively high concentrations of cortisol. The impairment of cardiovascular function cannot be ascribed to corticosteroid insufficiency. Su-4885, in concentrations similar to those estimated to exist in the body fluids of these dogs, exerted a negative inotropic response in isolated cat papillary muscle preparations.

1. Chart, J. J., Sheppard, H., Allen, M. J., Bencze, W. L., Gaunt, R., *Experientia,* 1958, v14, 151.

2. Chart, J. J., Sheppard, H., *J. Med. Pharm. Chem.,* 1959, v1, 407.

3. Jenkins, J. S., Meakin, J. W., Nelson, D. H.,

Endocrinology, 1959, v64, 572.

4. Stone, H. L., Ph.D. Thesis, Univ. of Illinois, 1961.

5. Lefer, A. M., Ph.D. Thesis, Univ. of Illinois, 1962,

6. Sutfin, D. C., Lefer, A. M., *Med. Electronics Biol. Eng.*, 1963, v1, 371.

7. Tanz, R. D., Kerby, C. F., *J. Pharmacol. Exp. Therap.*, 1961, v131, 56.

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Pharmacological Induction of Fibrinolytic Activity in the Dog.* (28913)

BURKHARD RAHN AND KURT N. VON KAULLA

Department of Medicine, University of Colorado School of Medicine, Denver

Activation of the fibrinolytic system with the aim of inducing thrombolysis can be produced in man by intravenous injection of exogenous material such as activator-free plasmin(1) or plasminogen activators, streptokinase and urokinase(2). The use of the body's own activators for thrombolysis has been poorly explored, though it has been repeatedly demonstrated that a variety of stimuli including pharmacological agents induce in man a marked rise of fibrinolytic activity. This is thought to be due to release of activator into the blood stream. Detailed exploration of this phenomenon has not been undertaken, primarily due to the lack of an appropriate experimental animal. In this paper it will be shown that pharmacological stimuli induce marked fibrinolytic activity in the dog.

Material and methods. Measurement of fibrinolytic activity. The lysis time of the euglobulin fraction prepared from citrated plasma was measured(3). Siliconized glassware was employed throughout the study. An intravenous catheter was inserted through a 14 G needle (Venocath™-14 Surgical 15 G, 8½'''', Abbott) into the jugular vein, and kept open by a slow saline infusion. The blood was withdrawn by the 2-syringe technique into a 5 ml syringe containing 1 ml of 3.8% sodium citrate USP. Tests were carried out within 45 minutes after shedding of the blood. Prolonged storage at any temperature tended to produce erratic results. The euglobulin lysis times were checked several times before and serially after i.v. injection of the compounds to be tested (Fig. 1A and 1B).

Animals and animal care. Healthy dogs of various breeds were obtained from the university-owned animal farm. In the laboratory the animals were kept for a few days in individual cages before the experiment. A diet consisting of either Frisky's canned dog food or of dog pellets mixed with horse meat and gravy was given.

Anesthesia. After taking an initial blood sample, all dogs were anesthetized with 26.5 mg/kg Diabutal® (Pentobarbital) intravenously. If required, additional smaller amounts of the anesthetic agent were given during the test. The anesthetized animal was lying on its back on a V-shaped dog board throughout the study.

Blood pressure recordings. After inserting a 16 G catheter into the femoral artery, the blood pressure and pulse rate were measured with Statham Strain Gauge (P23A) and visualized on a screen of a Hathaway Type MBC-2 oscillograph and recorded by a Heiland oscillogram on photo-sensitive paper.

Drugs. Carbachol (Delta Chemical Works, New York) 0.002% in saline, epinephrine (Parke, Davis, Detroit) 0.01%, and atropine sulfate (Mann Research Lab., New York) 10% in saline were used. The calculated doses were given intravenously in 60 seconds.

Results. Normal euglobulin lysis times. The average euglobulin lysis time before anesthesia in 22 healthy non-anesthetized dogs was 95 minutes, with variations from animal to animal as follows: 56, 64, 70, 72, 82, 82, 85, 88, 91, 95, 95, 95, 100, 100, 101, 101, 106, 108, 113, 116, 123, 142 minutes, S.D.

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