

Activation, Inhibition and Reversal of Inhibition of Plasma Volume Changes in Adrenalectomized and Intact Dogs.* (32213)

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These experiments concern: 1) the effectiveness of adenosine triphosphate (ATP) for raising the plasma volume (PV) of dogs possessing functional adrenals but non-effect upon animals lacking these glands; 2) the marked PV-raising action of ATP in adrenalectomized dogs when it is injected along with small but ineffective doses of glucocorticoid *per se*; 3) complete inhibition of such induced elevation of PV in either type of test animal by the cardiac glycoside G-strophanthin or calcium; 4) prompt reversal of the inhibition by administering salts of K, Mg and Na accompanied by sharp increase in PV.

Materials and methods. All injections and blood withdrawals were *via* the jugular veins. The 16 animals used, which lacked adrenals, had been operated for approximately 3 years and studied through numerous cycles of adrenal insufficiency and recovery on glucocorticoid therapy. In the 25-35 day interim between experiments during this long period they were maintained active, vigorous and in normal health by daily i.m. injections of desoxycorticosterone acetate in oil (DCA) plus ample salt supplements in the food. Hence, the dogs rarely received substitution therapy consisting solely of mineralocorticoid and salt for more than a few weeks at a time. Longer periods usually resulted in slow decline in plasma volume. Fasting, control, blood samples were taken at 9:30 a.m., the dogs were then fed and 24 hours later the material under investigation was injected i.v., water withheld and plasma volume, blood and plasma constituents determined. Control and experimental blood samples were generally taken within 50 minutes or less of one another; in all cases there was a definite waiting

period after the injectate was administered.

Plasma volume was determined using the dye T 1824 according to methods recommended by Gregersen and Rawson(1) and Shu Chien and Gregersen(2) and has been adequately referred to elsewhere by the writers(3), including the method for solubilizing the free alcohols of the steroids used for i.v. injection.

Results. Table I A and B record the effect of i.v. administration of 25 mg of prednisolone (Schering) upon the PV of intact and adrenalectomized dogs. The steroid had no effect on the PV when taken 50 minutes after injection in animals lacking adrenals. However, the same dosage given to the intact animal raised the PV an average of 22.5% above control values (Table I A and B). Blood constituents did not exhibit significant changes in the adrenalectomized dog but a modest decline in hemoconcentration accompanied the volume rise in the intact animal.

Table I C and D. Intravenous injection of 100 mg of ATP had no effect on the PV of animals without adrenal cortices but markedly increased the plasma volume of dogs possessing functional glands. Thus the same dose of ATP given to the intact dog promptly raised the PV 20.3% 20-50 minutes after administering the compound (Table I D). There was some decrease in hemoconcentration associated with PV increase. The ATP was obtained fresh from Nutritional Biochemicals Corp. and kept under refrigeration until a few minutes before using. Each 100 mg was prepared in 4 ml of water and given slowly by vein over 5-7 minutes in order to prevent untoward reactions such as retching.

Table I E, F, G. Twenty-five mg of glucocorticoid (prednisolone) inactive *per se*, plus 100 mg of ATP sharply raised the PV of ad-

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TABLE I. Plasma Volume Changes Induced in Adrenalectomized and Intact Dogs by i.v. Injection of Adenosine Triphosphate *per se* and in Combination with Glucocorticoids, Mineralocorticoids and G-Strophanthin.

Time after injection	Body wt (kg)	Blood urea N (mg %)	Blood sugar (mg %)	Hb (g %)	Hct (%)	Plasma volume (ml/kg)	Change (%)
A. Adrenalectomized. 25 mg prednisolone. Avg 7 dogs.							
Control	22.86	17.2	79.8	13.23	37.7	49.0	
50 min	22.83	15.0	78.6	13.01	35.0	47.8	— 2.4
B. Nonadrenalectomized. 25 mg prednisolone. Avg 13 dogs.							
Control	24.32	14.5	76.1	17.03	44.1	48.7	
50 min	24.27	14.1	80.3	14.14	42.7	59.7	+22.5
C. Adrenalectomized. 100 mg ATP. Avg 6 dogs.							
Control	23.88	17.5	82.1	12.49	32.9	48.9	
50 min	24.25	17.9	80.5	12.12	31.0	48.4	— 1.0
D. Nonadrenalectomized. 100 mg ATP. Avg 9 dogs.							
Control	22.35	13.8	80.1	16.46	43.0	51.1	
20-50 min	22.51	12.7	78.8	14.67	40.4	61.5	+20.3
E. Adrenalectomized. 25 mg prednisolone and 100 mg ATP. Avg 14 dogs.							
Control	22.04	17.0	74.8	14.00	37.6	46.0	
50 min	21.27	15.1	76.2	13.02	33.5	53.9	+17.1
F. Adrenalectomized. 25 mg desoxycorticosterone and 100 mg ATP. Avg 6 dogs.							
Control	23.04	19.0	76.2	13.96	39.0	45.0	
50 min	23.07	19.1	82.5	14.14	36.0	44.4	— 1.3
G. Adrenalectomized. 25 mg aldosterone and 100 mg ATP. 1 dog.							
Control	23.64	24.5	75.0	13.19	37.3	49.1	
50 min	23.52	20.0	80.0	13.35	34.9	47.2	— 3.8
H. Adrenalectomized. .02 mg/kg G-strophanthin, 25 mg prednisolone, 100 mg ATP. Avg 6 dogs.							
Control	22.82	20.1	71.8	13.46	37.5	46.9	
50 min	23.02	19.5	68.2	13.77	35.4	44.4	— 5.3
I. Nonadrenalectomized. .02 mg/kg G-strophanthin and 100 mg ATP. Avg 5 dogs.							
Control	24.07	15.9	84.0	15.08	44.0	49.3	
20 min	23.96	15.0	80.1	15.98	46.9	46.0	— 6.6
J. Nonadrenalectomized. .02 mg/kg G-strophanthin and 25 mg prednisolone. Avg 4 dogs.							
Control	25.05	16.1	84.8	16.70	43.8	49.4	
50 min	24.79	16.1	82.7	16.96	46.0	46.7	— 5.4

renalectomized dogs when administered i.v. within a few minutes of one another and the plasma volume determined 50 minutes later. Data in Table I E show that the combined injectate of steroid and ATP induced an average rise in PV of 17.1% in 14 dogs. Hemo-concentration decreased somewhat. Negative results were obtained when the solubilized free alcohols of the mineralocorticoids, aldosterone and desoxycorticosterone were given along with the ATP. Only one dog was used in the aldosterone test owing to lack of material (Tables I F and G).

Table I H and I. These data are concerned with the effect on the PV of adding a known inhibitor of adenosine triphosphatase (ATP-ase) to the prednisolone-ATP injectate. The

cardiac glycoside G-strophanthin was chosen because of its suppression of the activity of ATP-ase, the enzyme located in cell membranes, thought to be responsible for hydrolysis of ATP and release of its high energy phosphate bonds, considered by many to be the source of metabolic energy for maintaining the "sodium pump" (4,5). Data on 6 adrenalectomized dogs are presented in Table I H. In an earlier experiment, similar operated dogs had responded to the steroid plus ATP injection by an elevation of the PV of 17.1% (Table I E). Following the glycoside injection, no increase in PV occurred and inhibition was complete. G-strophanthin exhibits toxic properties and will induce retching and vomiting within a few minutes after i.v.

TABLE II. Inhibition of Plasma Volume-Raising Action of Adenosine Triphosphate and Adrenal Glucocorticoids by G-Strophanthin and Calcium Lactate; Reversal of Inhibition by Injections of 10 ml of 7.5% Solutions of KCl, MgSO₄ and NaCl.

Injectate—i.v.	Sampled min after injection	Body wt (kg)	Hb (g %)	Het (%)	Plasma volume	
					(ml/kg)	Change (%)
A. G-strophanthin inhibition of P V increase reversed by KCl. Avg 5 Adx† dogs.						
None	Control	22.54	13.71	36.3	43.6	
ATP, prednisolone, G-s*	50 min	22.53	13.22	35.2	44.0	+ .9
KCl	60 "	22.34	11.84	32.9	59.0	+34.0
B. Ca lactate inhibition of P V increase reversed by MgSO ₄ . Avg 4 Adx dogs.						
None	Control	25.63	12.77	43.9	44.2	
ATP, prednisolone, Ca lactate†	50 min	25.35	11.97	31.2	42.9	— 2.9
MgSO ₄	60 "	25.25	11.33	30.6	51.6	+20.2
C. Ca lactate inhibition of P V increase reversed by MgSO ₄ . Avg 4 intact dogs.						
None	Control	25.93	16.79	44.3	47.5	
ATP, Ca lactate	50 min	25.74	15.95	43.0	43.0	— 9.4
MgSO ₄	60 "	25.54	15.12	43.2	57.6	+33.9
D. Ca lactate inhibition of P V increase reversed by KCl. Avg 4 intact dogs.						
None	Control	27.44	15.88	44.5	49.4	
ATP, Ca lactate	50 min	27.24	16.21	44.0	48.2	— 2.4
KCl	60 "	27.04	14.62	42.3	60.5	+25.5
E. Ca lactate inhibition of P V increase reversed by NaCl. Avg 3 Adx dogs.						
None	Control	24.03	15.57	40.4	41.5	
ATP, prednisolone, Ca lactate	50 min	23.93	14.53	36.6	42.5	+ 2.4
NaCl	60 "	23.90	12.33	33.1	53.6	+26.1

* G-s = 0.02 ml/kg G-strophanthin.

† 10 ml of 7.5% solution Ca lactate injected slowly (6-7 min). All salts used were injected at this rate.

‡ Adx = adrenalectomized.

injection unless it is given very slowly over at least 6-7 minutes. However, doses of 0.02 mg/kg in 4 ml of distilled water rarely elicited symptoms of distress when given as suggested and appeared to exert maximum inhibitory effects on the PV 30 minutes after administration.

Table II presents data pertaining to the failure of 100 mg of ATP to raise the plasma volume of intact dogs when also injected with G-strophanthin. Animals possessing functional adrenals readily react to ATP *per se* 20-50 minutes after injection by marked rise of the PV (Table I D) but when given 0.02 mg/kg of the glycoside the PV failed to rise. The glycoside also prevented the PV rise in nonadrenalectomized dogs given 25 mg of prednisolone (Table I J). Such animals promptly increased the PV by 22.5% when the glycoside was omitted from the injectate (Table I B).

Table II shows that G-strophanthin and Ca salts antagonize the PV-raising activity of ATP, possibly by inhibiting the functioning of

the cellular membrane (Na⁺ — K⁺)-activated enzyme (ATP-ase) system. This inhibition is known to be reversible by addition of certain salts to the media, *e.g.*, MgSO₄(4,5), NaCl(6), and KCl(7,8).

Table II A to E reveals that G-strophanthin and calcium inhibition of the PV-increase in adrenalectomized dogs induced by glucocorticoids plus ATP and in intact animals by either substance administered singly, is also completely reversed by K, Mg and Na salts 60 minutes after injection. Plasma volume increases of 20-34% over control values were associated with reversal of the glycoside and Ca inhibition.

Discussion. Drury and Szent-Gyorgyi(9) demonstrated a general arterial and coronary vasodilation by adenosine compounds; since then others have confirmed and extended their findings. Adenosine triphosphate (ATP) markedly dilates the coronary(10,11), muscular(10,12,13), intestinal and cutaneous vessels(10), of rabbits, cats and dogs. Folkow (10), indicated that the vasodilating action of

ATP is exerted directly upon the blood vessels since denervation failed to diminish their vasoreactivity. Frohlich(14), observed that local infusion of sodium salts of adenosine mono-, di-, and triphosphate into the dog's forelimb greatly decreased the resistance to blood flow in the small vessels and that the decreased resistance was due primarily to arteriolar dilation. Thus ATP markedly increases blood flow at the periphery of the vascular tree, possibly by a direct vasodilating action upon the vasculature, thereby affording at least a partial explanation for the PV-raising action. However, the same dosage of ATP effective in dogs with functional cortices failed to induce PV change in animals lacking adrenals, which suggests the possibility that glucocorticoids may perhaps be necessary for activation or potentiation of the vasodilating activity of ATP. The fact that small doses (25 mg) of glucocorticoid *per se* are without effect upon the PV of dogs without adrenals but that such doses become highly effective when given along with ATP, lends plausibility to the suggestion. The vasodilating property of ATP is much greater when given intra-arterially rather than by vein(10). Unfortunately the present i.v. experiments were completed before this fact was appreciated.

The antagonism exhibited by G-strophanthin and calcium to the PV-raising action of ATP and adrenal glucocorticoids (Tables I and II), may possibly be related to the action of cardiac glycosides and calcium in suppressing the hydrolysis of ATP *in vitro* by reason of their antagonism to functioning of the sodium-potassium activated, magnesium-dependent adenosine triphosphatase (Na-K ATP-ase) located in cell membranes(4). According to students of active cation transport, when the activity of this enzyme is held in abeyance, pumping of Na and water out of cells ceases. G-strophanthin and calcium prevent the rise in PV which invariably follows glucocorticoid plus ATP injection in adrenalectomized animals and also of either steroid or ATP when administered separately to intact dogs. The inhibitory activity of the

glycoside and calcium on PV elevation *in vivo* and suppression of ATP-ase activity *in vitro* as demonstrated by enzymologists, is also readily reversed by KCl, MgSO₄ and NaCl. A satisfactory explanation for the experimental results discussed here requires further investigation.

Summary. Adenosine triphosphate raises the plasma volume of dogs possessing adrenal cortices but is without effect in 100 mg doses on the plasma volume of dogs lacking these glands. The negative action of adenosine triphosphate and also that of small (25 mg) ineffective doses of prednisolone *per se* upon the plasma volume of adrenalectomized dogs when injected singly, becomes highly positive when the two are administered together. These induced elevations of plasma volume in either type of test animal are completely inhibited by G-strophanthin and calcium. Prompt reversal of the inhibition follows administration of salts of K, Mg and Na and is accompanied by sharp increase in plasma volume.

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