Effects of Injection of 5-Adenylic Acid on Tissue Nucleotides of the Rat. (20701)

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Intramuscular injection of 5-adenylic acid in man produces significant shifts in the different nucleotide fractions present in the red cells, but no significant variation in the total nucleotide with doses between 20 and 100 mg(1). The present paper reports data on the alteration of adenine nucleotide levels in brain, muscle, liver, heart, and kidney of the rat in control animals and in animals injected with 5-adenylic acid and the localization of 5-adenylic acid labelled with radioactive phosphorus in various tissues.

Procedure. Adult, white, male rats weighing between 125 and 500 g were employed. No significant differences were observed with different batches of rats. The animals were injected with either 10 or 100 mg of 5-adenylic acid as the sodium salt,* intraperitoneally. Approximately 50 minutes after injection and 10 minutes before sacrifice, the animals were anesthetized with nembutal intraperitoneally (5 mg/100 g body weight). One hour after nucleotide injection, the skin surrounding the gastrocnemius muscle was carefully dissected free. The connective tissue sheath around the muscle was cut and the blunt end of a scalpel inserted beneath the connective tissue sheath freeing the muscle but allowing it to remain attached at its origin and its insertion. The animal was allowed to recover from the shock of the operation for 10 minutes, at which time the head was immersed in liquid nitrogen. While the skull was freezing, the gastrocnemius muscle was rapidly removed and transferred to liquid nitrogen. The time required for this manipulation varied between about 10 and 30 seconds. After the skull was frozen, the brain was removed without being allowed to thaw and also immersed in liquid nitrogen. Brain and muscle were then pulverized and deproteinized with trichloracetic acid. Liver, kidney, and heart, in the earlier experiments, were removed in the frozen state after the entire animal was frozen in liquid nitrogen. In later experiments, the tissues were removed from anesthetized animals and immediately plunged into liquid nitrogen. The results obtained with these two procedures were not significantly different. These tissues were also pulverized and deproteinized with trichloracetic acid. Tissues from control animals not receiving the 5-adenylic acid were treated in similar fashion. In the case of brain and muscle, the protein-free trichloracetic acid extracts were immediately analyzed for inorganic orthophosphate and phosphocreatine phosphorus according to the procedures described by LePage, in Umbreit, Burris, and Stauffer(2). The trichloracetic acid extracts remaining after carrying out these determinations were treated with barium and alcohol. The barium-alcohol insoluble precipitates resulting were collected by centrifugation, freed of barium and assayed for Adenylic Acid (AA), Adenosine Triphosphate (ATP), and Adenosine Diphosphate (ADP), enzymatically according to the procedure of Albaum and Lipshitz(3). Total purine was also calculated as adenine from the absorption in the Beckman spectrophotometer at 2600 Å. Some blood nucleotide determinations were also carried out on separate animals following the injection of 5-adenylic acid intraperitoneally. The blood was removed under heparin by cardiac puncture. AA, ADP, ATP, and total purine were measured on perchloric acid filtrates prepared as described previously(1). For the *tracer experiments,* adenylic acid with the phosphorus labelled as P^{32} was prepared by a fermentation procedure. The injected dose, 50 mg, contained 784,000 counts/minute. This was injected intraperitoneally and the animals were sacrificed under anesthesia at the end of one hour.

* Supplied as My-B-Den by the Ernst Bischoff Co. Inc., Ivoryton, Conn.

TABLE I. Phosphocreatine Levels of Brain and Muscle and Adenine Nucleotide Levels of Brain, Muscle, Heart, Liver, and Kidney Following Injection of 100 mg 5-adenylic Acid Intraperitoneally. (Animals sacrificed 1 hr after injection; adenine nucleotide levels expressed as mg % adenine; all values are mean values.)

	Brain		Muscle		Heart		Liver		Kidney	
	C (39)†	E (19)	C (41)	E (20)	C (11)	E (9)	C (11)	E (8)	C (12)	E (9)
PC	50.0	70.8*	218.2	264.5*						
AA	5.4	3.9*	2.1	1.1	13.3	10.9	11.4	11.3	9.5	9.7
ADP	1.4	1.1	1.2	2.4	5.5	4.6	5.9	4.6	3.5	3.3
ATP	14.2	21.8*	42.5	54.5*	32.6	41.1*	14.7	26.6*	12.0	11.7

* Difference between mean values of Control (C) and Injected (E) statistically significant. Statistical significance concluded if difference between means exceeds S.E. diff. by more than 2×. S.E. diff. $= \sqrt{(S.E._C)^2 + (S.E._E)^2}$.

† The figures in parentheses indicate the No. of different analyses performed.

TABLE II. Radioactivity in Various Tissues Following Injection of 50 mg of 5-adenylic Acid Intraperitoneally. (Animals sacrificed at the end of 1 hr.) Total counts/min. injected = 784000.

Tissue	Counts/min./mg* fresh wt
Adrenal	337
Liver	285
Kidney	195
Heart	145
Spleen	98
Blood	52
Testis	16
Muscle	16
Brain	11

* Each value is avg of 6 determinations.

TABLE III. Total Purine Content of Various Tissues Following Injection of 100 mg 5-adenylic Acid Intraperitoneally. (Animals sacrificed 1 hr after inj.; total purine values calculated as mg % adenine from 2600 Å reading.)

Tissue	n	Control	n	Exp.
Brain	7	35.8	21	39.3
Muscle	7	55.8	21	60.4
Heart	10	76.1	9	76.0
Liver	10	66.1	9	81.8*
Kidney	10	48.1	9	48.8

* Difference between means statistically significant.

On removal, the tissues were dissolved in 30% KOH and aliquots were transferred to planchets. These were dried and counted.

Results. After the injection of 10 mg, the only statistically significant result was a lowering of the AA of both muscle and brain. 100 mg of 5-adenylic acid, on the other hand, produced a marked increase in the adenosine triphosphate and phosphocreatine in muscle and in brain, without any significant change in the total purine (Tables I, III). Increases in ATP of human blood without alteration of the total purine level have been shown previously(1).

This indirect effect of the injected nucleotide on the purine "reservoir" prompted us to investigate the distribution of adenylic acid labelled with radioactive phosphorus in other tissues. The results are shown in Table II. On a weight basis, the adrenal gland concentrates the isotope more than any other tissue, followed by liver, kidney, and heart. Brain and muscle accumulate only small quantities.

Because of these results, we carried out nucleotide analyses on liver, kidney, and heart. Table I shows that there was no effect on the kidney nucleotides of injected 5-adenylic acid which suggests that the tracer is probably in the process of being eliminated. Liver and heart, however, showed significant increases in their ATP level (Table I). One point merits special emphasis: the heart showed no increase in total purine so that the alterations observed probably represent shifts in a nucleotide "reservoir". On the other hand, liver shows an increase in total purine which is statistically significant (Table III).

TABLE IV. Blood Nucleotides in the Rat. Mean values as mg % adenine.

	Control (n = 12)	100 mg AA inj.† (n = 16)
AA	.2	.7*
ADP	.7	.6
ATP	4.1	5.1*
Total purine	8.9	10.6*

* Difference between means statistically significant.

† Blood drawn ½ to 1 hr after inj.

The results on the rat blood removed after one hour are shown in Table IV. The concentration of known nucleotides and total purines is lower in rats than it is in mice and in men. The injection of adenylic acid produces a small but significant increase in the adenosine triphosphate level of the rat blood, and a corresponding increase in the total purine. Whether this difference between the behavior of the blood of rat and man is related to some peculiarity of the rat or whether this is related to the dose of adenylic acid injected, is not clear at the present time.

Discussion. On the basis of our original blood work(1), we had concluded that the effect of injected 5-adenylic acid on the blood was not a direct one, since the magnitude of the effect observed was, in most cases, in excess of what might be expected on the basis of the amount of adenylic acid injected. The fact that we have also observed an increase in the phosphocreatine level in brain and in muscle supports this conclusion. The conclusion is also supported by experiments in which it has been demonstrated that injected adenylic acid lowers the cholesterol levels, and produces alterations in the serum lipoprotein of experimental animals(4,5). All of these diversified effects produced by the injection of the nucleotide make it appear that the effect is not a simple one, but is rather complex. This, coupled with our observation that the adrenal gland accumulates radioactive phosphorus from injected adenylic acid in much greater concentration than any other tissue, opens some interesting possibilities with respect to this organ being implicated in some of the observed effects.

As our data show, not all organs show alterations in tissue nucletoide on the injection of 5-adenylic acid; for example, kidney. Certain tissues like tumors(6), on the other hand, as we reported earlier, show a significant decrease in nucleotide.

Summary. 1. Tissue nucleotide levels (AA, ADP, ATP, and total purine) of control rats and those injected with 5-adenylic acid have been studied. At the 100 mg injection dose, brain, muscle, liver, and heart show significant increases in adenosine triphosphate. Of these 4 tissues, only liver exhibits a measurable increase in total purine. Kidney shows no effect. The cellular element of blood shows a small but significant increase in adenosine triphosphate, but this is accompanied by a corresponding increase in total purine. 2. The experiments in which 5-adenylic acid, containing radioactive phosphorus is injected into rats shows a maximum accumulation of the label on a weight basis in the adrenal gland followed by liver, heart, kidney, spleen.

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