PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 544–549 (1975)

The Synthesis of Taurine from Sulfate VIII. The Effect of Potassium[1] (38580)

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The synthesis of taurine from serine and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) by the PAPS-sulfotransferase (EC 2.8.2) (P_1) has been reported (1). During the initial phases of this research the enzyme fraction was dialyzed for 18 hr against cold water. Subsequent experiments indicated that the P_1 activity was enhanced by the addition of potassium ion (1). When the P_1 enzyme was dialyzed extensively (48–72 hr), the P_1 activity was lost and could not be regained by the addition of pyridoxal phosphate, a sulfhydryl reagent or any of several monovalent or divalent ions (2).

During the past few years, interest in the function of tissue taurine has become intense. This has been especially so in cardiac studies since the concentration of taurine in this tissue is high in all animals (3). The relationship between taurine and the key ions in muscle and nervous cellular activities has been studied by numerous investigators. Read & Welty (4) demonstrated that taurine can prevent the loss of cellular K^+ and thereby may depress cardiac manifestations of hyperirritability.

These experiments were conducted, using the P_1 enzyme system from rats, to ascertain the influence of K^+ on the *in vitro* and *in vivo* activity of PAPS-sulfotransferase.

Material and Methods. The rats used in these experiments were young albino males from an inbred colony maintained by the Agricultural Biochemistry faculty at W.V.U., or from Hilltop Laboratory Animals, Scottdale, PA. The rats were fed a purified diet composed of: Isolated soybean protein, 20%; DL-methionine, 0.3%; Wesson Oil, 3%; complete mineral and vitamin premix, 10%; and glucose monohydrate, 66.7%. The mineral premix contained the macro and micro elements except potassium in the quantity recommended by the NRC (5). Potassium chloride was omitted from the mineral premix so that diets with variable levels of K^+ could be prepared. The control diet contained 0.2% K^+.

The rats were fed the complete diet for three weeks, sacrificed by stunning and decapitation, and the heart and liver were excised. The tissues were washed with cold distilled water and frozen. The tissues were homogenized, fractionated and the PAPS-sulfotransferase (P_1) was assayed as previously described (1). The P_1 spec act is the cpm^{35}S-taurine formed from PAP^{35}S per min per mg protein. The PAP^{35}S was enzymatically synthesized (6), the protein concentration determined by the method of Lowry *et al.* (7), and the tissue taurine determined by the Pentz technique (8).

A series of *in vitro* experiments was conducted to ascertain the influence of K^+ on the P_1 activity of rat heart and liver. These experiments were designed to test the theory that the K^+ concentration of the tissue could regulate the *do novo* synthesis of taurine from sulfate, and that the enzyme activity and stability was specific for K^+.

The P_1 enzyme was dialyzed by placing 30 ml of the P_1 fraction in cellulose tubing and suspending in 4 liters of deionized H_2O at 5°. The dialysis was continued for varying periods of time. The P_1 protein was dialyzed at 24, 48, and 72 hr and the P_1 activity was then determined.

The influence of K^+ on the P_1 activity was determined by the addition of KCl to the nondialyzed P_1 enzyme as well as the dialyzed protein. Potassium chloride was then added to the deionized H_2O during the dialysis and the P_1 activity was subsequently measured. To ascertain the specificity of the

[1] This manuscript is published with the approval of the Director of the West Virginia Agricultural Experiment Station, Morgantown, as Scientific Paper No. 1340.

[2] This work is in partial fulfillment of the requirements for the degree of M.S. in Agricultural Biochemistry, West Virginia University.

enzyme for K^+, a P_1 fraction was dialyzed for 24 hr and aliquots were added to the reaction mixture which contained 100 μmoles of K^+, Na^+, Li^+, NH_4^+, Mg^{2+}, or Ca^{2+}. Each of these elements was added as the Cl^- salt.

An *in vivo* experiment was designed to measure the effect on the P_1 enzyme activity of the dietary level of K^+ and the taurine concentration of rat liver and heart. Eight rats were randomly assigned to one of three dietary groups containing 0.1, 0.2, or 0.4% K^+. These diets were pair-fed for 4 wk at which time the rats were weighed, and each rat was subcutaneously injected with 20 μCi $^{35}SO_4^=$. The rats were sacrificed 4 hr after injection and the hearts and livers excised. Total taurine, taurine spec act, and the P_1 activities were determined for each rat of each dietary group.

To ascertain that the P_1 specific activity was a reflection of the K^+ concentration, the P_1 fraction obtained from the rats fed the 0.1% and the control (0.2% K^+) diets were then reassayed after 100 μmoles of K^+ was added.

Results. The P_1 sulfotransferase specific activity of rat heart and liver was shown to vary with the K^+ concentration of the reaction media (Fig. 1). The P_1 protein from heart and liver showed the same response to K^+, although the spec act of the liver was higher. In each case, when 100–120 μmoles of K^+ was added to the reaction mixture containing 200 μg protein, optimal spec act was obtained.

The P_1 protein was dialyzed for 24, 48, or 72 hr and the spec act determined (Table I).

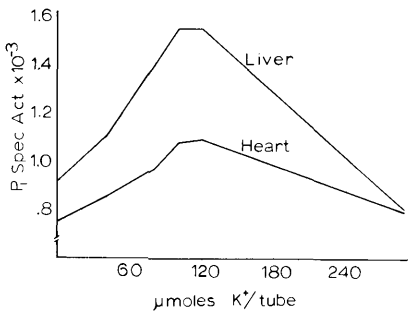

FIG. 1. The effect of varying the K^+ concentration of the P_1 assay mixture on the specific activity of rat heart and liver.

TABLE I. THE EFFECT OF DIALYSIS AGAINST DISTILLED WATER ON THE SPECIFIC ACTIVITY OF THE P_1 TRANSFERASE ENZYME OF RAT HEART.[a]

Hours dialyzed	Taurine ^{35}S (cpm/ml)	P_1 spec act	% Activity Lost
0	97,750	1649	0
24	10,600	223	87
48	4,347	100	94
72	0	0	100

[a] Each value is the mean of four determinations.

TABLE II. THE EFFECT OF K^+ ADDITION OF THE P_1 ENZYME SPECIFIC ACTIVITY OF RAT HEART AFTER 24, 48, AND 72 HOUR DIALYSIS.[a]

Hours dialyzed	μmoles K^+ added	Taurine ^{35}S (cpm/ml)	P_1 spec act
0	0	74,000	1550
24	10	18,714	393
	30	22,761	478
	50	38,714	813
	70	54,666	1148
	90	66,428	1395
	100	70,857	1488
	160	74,428	1563
	200	78,476	1648
	300	83,428	1752
	900	18,428	387
48	0	6,190	130
	10	5,142	108
	30	6,714	141
	50	5,857	123
	70	2,619	55
	90	6,380	134
	100	2,476	52
	200	8,047	169
72	100	1,380	29

[a] Each value is the mean of three determinations.

Dialysis for these periods caused the P_1 activity to be essentially lost. If, however, KCl was added to the dialyzed P_1 protein, the activity was restored if the preparation was dialyzed for only 24 hr (Table II). No restoration of P_1 activity was observed by adding K^+ to the preparation dialyzed for 48 or 72 hr.

This response of P_1 activity to K^+ suggested that the enzyme required the element in the role of allosteric modifier or cofactor,

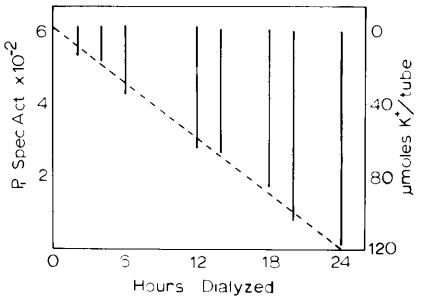

FIG. 2. The P_1 specific activity of rat heart after various dialysis times (dashed line) and the amount of K^+ required to restore the optimal activity of each (bars).

TABLE III. THE EFFECT OF K^+ ON THE P_1 SPECIFIC ACTIVITY OF RAT HEART SUBJECTED TO DIALYSIS.[a]

Dialysis Time (Hr)	µmoles K^+/ml water	Taurine ^{35}S (cpm/ml)	P_1 spec act
0	0	26,279	444
24	0	10,150	178
	60	26,011	450
	120	49,132	850
48	0	4,140	84
	60	26,589	460
	120	50,111	867
72	0	0	0
	60	24,971	432
	120	50,289	870

[a] Thirty ml of the P_1 enzyme was dialyzed in liters of water containing 0, 60 or 120 µmoles/ml of K^+.

and that the ions were being removed by dialysis. When the P_1 enzyme fraction was dialyzed for varying times from 0 to 24 hr and then graded levels of K^+ added to each, the restoration of activity shown in Fig. 2 was observed. In most instances, dialysis for longer than 24–28 hr resulted in irreversible denaturation.

Verification of the K^+ specificity was observed when the P_1 protein was dialyzed in distilled H_2O containing 0, 60, or 120 µmoles of K^+ (Table III). Without K^+ the P_1 activity was lost, but with 60 or 120 µmoles the activity was retained and stable even after 72 hr dialysis.

Having established that the PAPS-sulfotransferase was K^+ specific, an experiment was conducted to ascertain whether it was specific for that ion rather than for other monovalent or common divalent ions. When the P_1 activity was measured after dialysis for 24 hr in the presence of various ions, the only response was to K^+ (Table IV), indicating specificity for potassium.

TABLE IV. RECOVERY EFFECT OF VARIOUS IONS ON THE ACTIVITY OF THE P_1 24-HR - DIALYZATE.*, **

Hours dialyzed	Ions added[a]	Taurine ^{35}S cpm/ml[b]	P_1 spec act[b]
0	0	82,500	1650
24	0	10,000	200
	NH_4^+	10,500	210
	Na^+	10,250	205
	Li^+	13,100	262
	Mg^{2+}	10,000	200
	Ca^{2+}	10,000	200
	K^+	75,422	1734

[a] The ions were added to provide 100 µmoles/ reaction mixture in the chloride form.

[b] Each value is the mean of two determinations.

TABLE V. EFFECT OF DIETARY K^+ ON THE ACTIVITIES OF THE HEART AND LIVER P_1 TRANSFERASE ENZYME.

Tissue*	Dietary K^+	Taurine ^{35}S (cpm/ml)	P_1** spec act
Heart	0.1	32,900	570 ± 2[a]
	0.2	43,652	746 ± 3[b]
	0.4	32,975	588 ± 10[a]
Liver	0.1	26,153	510 ± 16[a]
	0.2	40,051	781 ± 11[b]
	0.4	32,051	625 ± 12[a]

* Eight individual livers were analyzed and hearts were analyzed by pooling four hearts giving two samples per diet.

** The values with different letters are significantly different when analyzed by Duncans multiple range test, ($P < .05$).

To establish the effect of dietary K^+ on taurine formation and concentration in the tissue, an *in vivo* experiment was then conducted in which three levels of K^+ were consumed by the rats for 4 wk (Table V). The P_1 spec act of the heart and liver was significantly depressed by the low dietary K^+ as

TABLE VI. THE EFFECT OF DIETARY K^+ ON LEVELS OF TOTAL TAURINE IN THE RAT HEART AND LIVER.

Tissue*	Dietary K^+ (%)	μg of taurine/g of tissue**
Heart	0.1	94.8 ± 2.4[a]
	0.2	152.4 ± 2.4[b]
	0.4	138.5 ± 1.5[c]
Liver	0.1	49.8 ± 4.9
	0.2	53.1 ± 3.3
	0.4	58.9 ± 2.6

* Eight individual livers were analyzed and hearts were analyzed by pooling four hearts giving two samples per diet.

** The values with different letters are significantly different when analyzed by Duncans multiple range test, ($P < .05$).

TABLE VII. RATE OF INCORPORATION OF $^{35}SO_4^=$ INTO HEART AND LIVER TAURINE 3 HR AFTER SUBCUTANEOUS INJECTION.

Tissue*	Dietary K^+ (%)	Taurine ^{35}S (cpm/ml)	Taurine Specific Activity**,***
Heart	0.1	700	7.35 ± 0.2[a]
	0.2	2075	13.60 ± 0.0[b]
	0.4	1043	7.50 ± 0.1[a]
Liver	0.1	1552	31.1 ± 1.9[a]
	0.2	2776	52.4 ± 5.0[b]
	0.4	1797	30.5 ± 2.7[a]

* Eight individual livers were analyzed and hearts were analyzed by pooling four hearts giving two samples per diet.

** Specific activity is defined as the cpm/ml taurine-s^{35} divided by the μg of taurine/mg of tissue.

*** The values with different letters are significantly different when analyzed by Duncans multiple range test, ($P < .05$).

compared to the normal level (0.2%), and was again depressed by the high dietary level of K^+.

The taurine concentration of the liver was not altered by the levels of dietary K^+, but the heart taurine was dramatically decreased by the K^+ deficiency (Table VI). When the rate of $^{35}SO_4^=$ conversion into heart and liver taurine was compared in these rats, a highly significant increase was observed in those animals fed the 0.2% K^+ diet over those fed the low and the high K^+ diet (Table VII).

The P_1 spec act of the heart from the rats fed the 0.1% and the 0.2% K^+ diets was determined, after which 100 μmoles of K^+ were added to the reaction mixture of each enzyme fraction. After addition of the K^+, the heart P_1 spec act of the rat receiving the low K^+ diet was increased from 222 to 339 while the activity observed in the heart of rats fed the 0.2% K^+ diet was changed from 308 to 297. These data strongly suggest that the ability of the animal tissue to synthesize taurine from sulfate is regulated by K^+ level in the cell.

Discussion. The observation that the PAPS-sulfotransferase (P_1) of chick liver was activated with KCl (1) has been extended to include rat heart and liver, and these data indicate a specific role for K^+ as an allosteric modifier. Since the P_1 enzyme appears to be a constitutive enzyme for animal tissues (9) and K^+ the common intracellular monovalent cation, these data establish a further interrelationship between cation and anion metabolism.

During the *in vitro* experiments, deactivation of the P_1 enzyme by dialysis was prevented by the presence of K^+. The ion was effective in stabilizing the protein during dialysis and in the restoration of P_1 activity which was lost during short dialysis. With longer dialysis the inactivation was irreversible and the protein preparation would become an opaque colloidal suspension.

Activity lost during dialysis for up to 24 hr was stoichiometrically regained by added K^+. It would appear that the activity of the stable enzyme, as well the reversibility of the denaturation due to short dialysis (up to 24 hr) is directly proportional to the environmental K^+ concentration. It has been reported by Read and Welty (4) that taurine will control the K^+ concentration of dog heart by prevention of leakage from the cell membrane. Kaczmarek and Davison (10) reported the rapid release of taurine from rat brain slices subjected to electrical shock. Tower (11) observed a significant increase in cellular and mitochondrial Ca^{2+} when cat cerebral cortex was treated with ouabain and that taurine gave protection against these ouabain-induced ionic changes. The NaK ATPase of cell membranes has been shown to be inhibited by a cardio glycoside such as ouabain, and this inhibition is re-

versed by taurine (11). Furthermore, this membrane ATPase activity has been shown to transfer 2 μmoles of K^+ and 3 μmoles of Na^+ across the membrane per μmole of ATP hydrolyzed (12). The ability of orally administered Ca^{45} to be absorbed, transported in plasma and absorbed by bone was shown to be decreased in chicks fed taurine (13). It therefore appears that taurine may function in association with the membranes of electrically excitable tissue such as muscle and nervous system.

Since the efflux of taurine may be determined by the fluxes of other small molecules (14), it was not unexpected to find that the rate of taurine biosynthesis was influenced by the dietary K^+ concentration (Table V). The observation of Table VI that the taurine concentration of heart was drastically decreased was significant and unexpected. Although the cellular K^+ concentration was not determined, the K^+ concentration of the plasma was significantly lower in rats fed 0.1% K^+ (3.6 mEq/liters) compared to those fed 0.2% K^+ (5.7 mEq/liters).

Liver, considered a non-excitable tissue, showed a similar response of P_1 activity to dietary K^+ as did the heart. This supported the view that the K^+ effect was a specific modifier-enzyme response and that the specific activity determined from both tissues would merely be a reflection of the available K^+ when that tissue was sampled. When the P_1 spec act was determined from heart obtained from rats fed the low and normal K^+ diets and determined again after 100 μmoles K^+ was added, the P_1 activity of the low K^+ heart was increased to that of the normal K^+ heart.

The enzyme system of animal tissues which synthesizes taurine from serine and PAPS has been called a constitutive enzyme due to its presence in all tissues tested (9). The activity of this enzyme, termed the inorganic pathway of taurine biosynthesis to distinguish it from the transsulfuration pathway where the origin of taurine-S is organic (15), is stable in a B_6-deficiency while the decarboxylase activity of the transsulfuration pathway is not (16). This alternate pathway of taurine biosynthesis in the rat (17) may therefore be of significance to the animal due to its universal presence, as compared to the limited tissue occurrence of the organic taurine pathway enzymes (18). Its regulation by members of the transsulfuration pathway (15) may be of significance in the intracellular regulation of the taurine concentration. Therefore, the dietary parameters of amino acids, vitamins and minerals appear once again metabolically interrelated at a critical juncture, the maintenance of membrane integrity of animal cells.

Summary. The *in vitro* and *in vivo* influence of K^+ on the activity of rat heart and liver PAPS-sulfotransferase (P_1) was tested. The P_1 activity was optimal with 0.5 μmoles K^+ per μg protein. The P_1 activity was decreased by mild dialysis and lost by extended dialysis. The enzyme was stable when dialyzed with K^+ and tests with other ions indicated K^+ specificity. Rats fed purified diets with low, normal or high levels of K^+ had significantly different heart taurine concentrations and P_1 specific activities. These P_1 specific activities became comparable to those from the rats receiving the normal K^+ diet by the addition of KCl to the assay mixture.

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Received September 10, 1974. P.S.E.B.M. 1975, Vol. 148.