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Adrenergic Stimulation of Taurine Transport by the Heart

Abstract. *A high-affinity transport system that is specific for β-amino acids has been delineated in rat hearts. This system transports the cardiotonic sulfonic amino acid taurine. β-Adrenergic stimulation increases the transport capacity without effect on α-amino acid uptake, as does stimulation with adenosine 3',5'-monophosphate or theophylline. The existence of such an uptake system for taurine in the heart accounts for the high intra- to extracellular concentration gradient that is maintained, and suggests that cardiac stress is associated with increased taurine uptake. This may explain why taurine is the only amino acid to be markedly elevated in congestive heart failure. Taurine is a modifier of calcium fluxes in the heart, as are β-adrenergic agonists. The presence of this uptake system suggests a link between β-adrenergic stimulation of calcium and taurine fluxes.*

One of the major mechanisms whereby the heart increases its output under work stress is the β-adrenergic system. Prolonged stimulation of this system causes an increase in heart mass—cardiac hypertrophy—and, if the stress is severe and long-lasting, eventual congestive heart failure. Stimulation of the β-adrenergic system thus causes the physiological changes summarized as the "fight or flight" syndrome, but a high level of stimulation for a prolonged period causes pathological changes leading to cardiac hypertrophy and decreasing cardiac efficiency. An understanding of the cellular changes occurring at different levels of β-adrenergic activation is therefore crucial for an elucidation of the biochemical basis for cardiac hypertrophy.

One of the major actions of agonists of the β-adrenergic system in increasing cardiac contractility is to increase the flux of calcium into the cell. Another modifier of calcium fluxes in the heart is the sulfonic amino acid taurine (*1*). Taurine is present in mammalian heart in large quantities, comprising in excess of 50 percent of the total free amino acid pool (*2*). The content of taurine in the heart is maintained remarkably constant, and in the rat is invariant over a wide range of conditions. It is of interest, therefore, that marked elevations in taurine concentration occur in congestive heart failure in humans and experimental animals (*3*). We have examined the relation between taurine content and cardiac stress, and now propose that a transport system for taurine exists in the heart that is modulated by the level of β-adrenergic activation. The existence of such a system may explain why the concentration of taurine is markedly elevated in hearts in the process of congestive failure.

The precise functions of taurine in the heart are undelineated, but taurine is known to alter the kinetics of calcium, leading to a decreased rate of exchange with extracellular fluid, and an increased affinity of intracellular structures for calcium (*2*). The concentrations of taurine attained in the heart are determined by the balance of any metabolic and transport processes that are operative. Although the rat heart can synthesize taurine, the quantitative importance of this is unclear (*4*). The only metabolic pathway for taurine for which evidence is available is conversion to isethionic acid (*5*). However, this pathway proceeds at a very low rate, or possibly is absent in the heart, and has no appreciable influence on taurine balance. No transport process has been shown for taurine in the intact heart, but in view of the fact that the myocardium/serum ratio of taurine is in excess of 200, it is likely that an active transport process exists. Such transport systems for taurine have been demonstrated in Erhlich ascites cells (*6*), platelets (*7*), and fractions of brain tissue enriched in glial and synaptosomal fragments (that is, crude synaptosomes) (*8*).

This study was designed to determine whether metabolic or transport processes affecting taurine concentration are modified by cardiac stress. We used isoproterenol, a β-adrenergic agonist, to produce a high output of stress on the heart. Preliminary studies in vivo revealed that isoproterenol given to rats for periods of up to 10 days produced a cardiac hypertrophy accompanied by a marked increase in total taurine content of the heart (*9*). No alteration occurred in the rate of taurine synthesis, as measured either by the overall conversion of cysteine to taurine, or by the activity of cysteamine dioxygenase (E.C. 1.13.11.19), an enzyme involved in the biosynthesis of taurine in the heart. Increases were observed, however, in the rate of influx of taurine. The effect of isoproterenol on taurine influx has therefore been examined in the isolated, perfused rat heart.

In the heart perfused by the Langendorff technique, the rate of taurine influx was constant for more than 20 minutes when taurine was perfused at concentrations of 25 to 200 μM (Fig. 1). [^{3}H]Taurine (specific activity, 1.4 mc/mmole) was perfused through the heart, and the radioactivity eluting from the heart was monitored (Fig. 1). Uptake was determined as the difference be-

Table 1. Effect of isoproterenol on amino acid influx in the heart. Data are expressed as the mean ± standard error of four hearts. The amino acids were perfused in 0.05 mM concentrations. Other conditions were as in Fig. 1 (*12.*).

Treatment	Influx [nanomole per gram (dry weight) per minute]				
	Taurine	β-Alanine	Aminoisobutyric acid	Leucine	Serine
Control	15.0 ± 1.0	16.0 ± 1.3	13.4 ± 1.3	28.5 ± 3.8	53.3 ± 3.5
Isoproterenol-stimulated	19.2 ± 0.2*	19.7 ± 1.0*	12.3 ± 0.5	30.6 ± 0.8	57.3 ± 1.3

*$P < .05$.

tween radioactivity entering the heart and radioactivity leaving the heart. The experimental design yields information concerning the rate of taurine influx, rather than rate of uptake, which is the algebraic sum of the rates of influx and efflux. Efflux rates were determined by perfusion with a taurine-free medium, the taurine in the eluate from the heart being measured with an amino acid analyzer. Isoproterenol did not affect this efflux rate. The initial fast rate of removal of [^{3}H]taurine shown in Fig. 1 was caused by the exchange of perfusate with interstitial fluid. Equilibration occurred within 20 seconds, after which the disappearance of radioactivity was due to cellular uptake. Perfusion with cold taurine in concentrations 50-fold greater than that of the radioactive taurine did not result in rapid displacement of the [^{3}H]taurine, as would have occurred if the radioactive taurine were bound to the external surfaces of the cells. Figure 2 depicts the Lineweaver-Burk plot of taurine influx into the heart. The process has a Michaelis constant (K_m) of 45 μM and a maximum velocity (V_{max}) of 32 nmole per gram of tissue (dry weight) per minute. This system is intermediate in affinity to the high- and medium-affinity transport systems observed elsewhere. Rat brain synaptosomes have been reported to have affinities (K_m) for taurine of 300 and 400 μM (*8*), platelets, affinities of 2.9 and 100 μM (*7*), and Ehrlich ascites cells, 150 μM (*6*).

Addition of isoproterenol to the perfusion medium resulted in an immediate stimulation of taurine influx. The stimulation in influx is dependent on the concentration of isoproterenol perfused over the range $7 \times 10^{-9} M$ to $4 \times 10^{-7} M$. Higher concentrations of isoproterenol caused a decreased stimulation of the rate of influx, and at $4 \times 10^{-5} M$, taurine influx was not increased over the control. This may be a toxic response due to the general cellular derangements and arrhythmias occurring at high concentrations of isoproterenol. The Lineweaver-Burk plot of taurine influx in the presence of $4 \times 10^{-7} M$ isoproterenol is shown in Fig. 2. The affinity is decreased to a K_m of 62 μM, and the V_{max} is changed to 42 nmole per gram (dry weight) per minute, a 40 percent increase. The double reciprocal plots of taurine and taurine plus isoproterenol are essentially parallel. A mechanism whereby the rate of formation of the taurine transport site complex was not affected after exposure to isoproterenol, but the rate of translocation of taurine from the transport site to the inside of the cell membrane was increased, would produce the kinetic plot shown in Fig. 2 in that, at a given concentration of taurine, in the presence of isoproterenol, the relative saturation of the transport site would be less (that is, a higher K_m) but the rate of transport higher (a higher V_{max}). Addition of isoproterenol to spontaneously beating, isolated, perfused hearts results in an increase in heart rate. Heart rate was discounted as being an influence on taurine influx by experiments on hearts where the chronotropic actions of isoproterenol had been eliminated by ablation of the sinoatrial node. Hearts paced at rates between 250 and 450 beats per minute showed no variation in taurine influx. Influx rates observed at 250, 350, and 450 beats per minute were 14.2 ± 0.1, 14.3 ± 0.7, and 13.6 ± 0.7 nmole per gram (dry weight) per minute, respectively, on perfusion with 50 μM taurine.

Stimulation of amino acid influx by isoproterenol was specific for β-amino acids (Table 1). When perfused at 50 μM

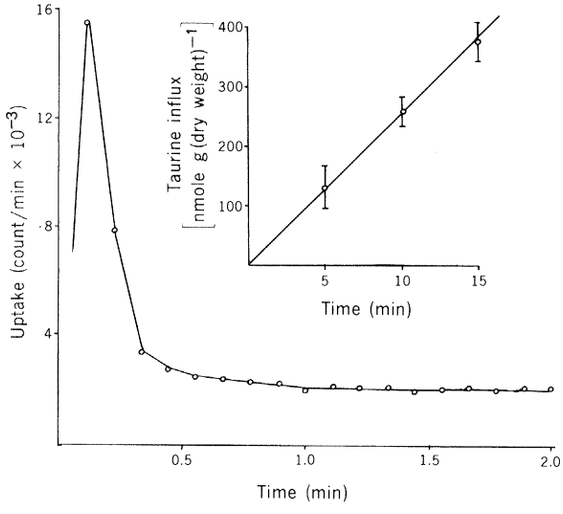

Fig. 1. The rate of uptake of [^{3}H]taurine by isolated perfused rat heart. Hearts from male rats weighing 200 g were perfused retrogradely by the Langendorff procedure with Krebs-Henseleit bicarbonate buffer at 37°C. The addition of $2.5 \times 10^{-5} M$ [^{3}H]taurine (specific activity, 1.4 mc/mmole) to the medium resulted in the removal of radioactivity from the perfusate. After 0.5 to 1.0 minute of perfusion, the rate of removal was constant, as illustrated. The inset shows that the rate of removal was constant for more than 15 minutes. Each point is the mean ± standard error from four hearts.

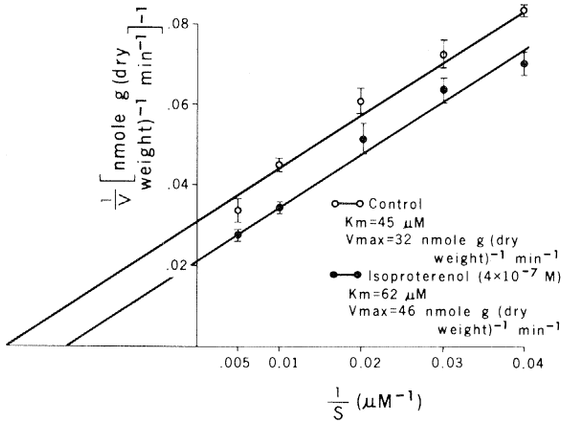

Fig. 2. Lineweaver-Burk plots of taurine influx in isolated male rat heart. Each point represents the mean ± standard error of four hearts. The hearts were perfused as in Fig. 1 in the presence or absence of isoproterenol. The addition of [^{3}H]taurine to the medium in concentrations between 2.5×10^{-5} to $20 \times 10^{-5} M$ resulted in a dose-dependent uptake by the heart. The rate of uptake was constant for more than 20 minutes. The lines were fitted by linear regression analysis. Symbols: ○, no isoproterenol; (●), $4 \times 10^{-7} M$ isoproterenol added.

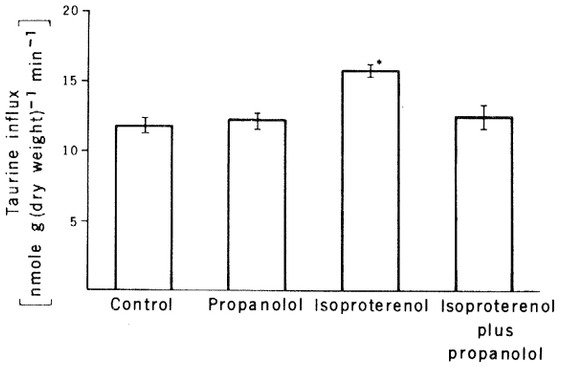

Fig. 3. the effect of propranolol on isoproterenol-stimulated taurine influx. The perfusion concentrations were taurine, $5 \times 10^{-5} M$; propranolol, $1 \times 10^{-5} M$; and isoproterenol, $4 \times 10^{-7} M$. Data are expressed as the mean ± standard error of four hearts per group. The asterisk indicates $P < .05$.

concentrations, taurine and β-alanine showed stimulated influxes of the same magnitude in the presence of isoproterenol, whereas leucine, serine, or aminoisobutyric acid (a transportable but nonmetabolizable amino acid) showed no change. Furthermore, α-amino acids did not influence the influx of taurine when perfused simultaneously with taurine at concentrations fivefold higher. β-Alanine under the same conditions inhibited taurine influx by 85 percent. The lack of competition between α- and β-amino acids indicates that transport occurs in the heart at separate sites, one site being specific for β-amino acids. Similarly, specific transport sites for β-amino acids have been shown to exist in Ehrlich ascites cells (*6*), and brain (*10*). Other workers have shown that increased influx of nonutilizable α-amino acids occurs in hearts under work stress (*11*). These increases were not observed until after 15 minutes of increased work load in isolated working hearts. The stimulation in β-amino acid influx reported here occurs within 20 seconds of exposure of the heart to isoproterenol, the first time interval at which we are able to determine influx rate.

The major pharmacological action of isoproterenol is that of β-adrenergic agonism. That the effect observed on the β-amino acids was indeed an adrenergic action was verified by the finding that propranolol blocked isoproterenol-induced stimulation of taurine influx (Fig. 3). However, perfusion of propranolol in the absence of isoproterenol did not result in a measurable change in influx of taurine. This suggests that there is one component of taurine influx in the heart which is independent of adrenergic influences and another component that is adrenergically modulated. Additional evidence for adrenergic involvement is shown in Table 2. β-Adrenergic effects are generally considered to be mediated intracellularly by the formation of adenosine 3′,5′-monophosphate (cyclic AMP). Perfusion of hearts with dibutyryl cyclic AMP resulted in a stimulation of taurine uptake of the same magnitude observed with isoproterenol. Theophylline, a phosphodiesterase inhibitor that decreases the rate of enzymatic hydrolysis of cyclic AMP, elevating endogenous levels, produced a similar result.

On the basis of our observations, we propose the following: A high-affinity uptake system for taurine exists in the heart which has a basal rate in the absence of adrenergic influences. In the presence of β-adrenergic stimulation this system is stimulated; the level of stimulation depending on the degree of β-adrenergic activation. At maximum stimulation, the system has 20 to 30 percent higher transport capacity than the noninduced system. The induction is cyclic AMP–dependent and possibly involves the phosphorylation of a membrane transport site. The elevated taurine concentrations found in the affected ventricles in congestive failure are explained by the presence of a modulated uptake system for taurine, maximally stimulated in a stressed heart in a state of chronic β-activation. In addition, this system provides a potentially important link between two agents that modulate calcium flux in the heart cell: β-adrenergic activation stimulates both calcium and taurine influx into the heart cell, and taurine modulates the pool size of free intracellular calcium.

Table 2. Effect of various agents on the rate of taurine influx. The concentrations were as follows: taurine, $1 \times 10^{-4} M$; isoproterenol $4 \times 10^{-7} M$; theophylline $1 \times 10^{-3} M$; and dibutyryl cyclic AMP $9.5 \times 10^{-4} M$. The control rate for the dibutyryl cyclic AMP experiment was 20.47 ± 0.24.

Treatment	Influx [nanomole per gram (dry weight) per minute]	Percentage of control
Control	20.01 ± 0.40	100 ± 2
Isoproterenol	23.96 ± 0.80*	119 ± 4
Theophylline	22.67 ± 0.03*	112 ± 1
Dibutyryl cyclic AMP	25.21 ± 1.73*	123 ± 8

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β-Endorphin: Endogenous Opiate or Neuroleptic?

Abstract. *The opiatelike neuropeptide β-endorphin produces a spectrum of effects that contrasts with that induced by the neuroleptic haloperidol. Rats injected intraventricularly or directly into the periaqueductal gray with β-endorphin (0.5 to 50 micrograms) exhibited rigid immobility accompanied by the loss of righting reflex; the period of rigidity was preceded or followed (depending upon dose) by a state of hyperactivity. In contrast, no dose of haloperidol tested (0.5 to 12 milligrams per kilogram) produced rigidity, loss of righting reflex, or behavioral excitation. Furthermore, whereas animals injected with haloperidol remained stationary on a vertical grid, rats injected with β-endorphin typically slid off the grid. Moreover, combined β-endorphin and haloperidol treatment produced flaccidity in most animals. These results do not support the contention that this opiatelike peptide may be a naturally occurring neuroleptic.*

Recently we reported that intraventricular administration of β-endorphin, which is β-lipotropin, residues 61 to 91 [β-LPH-(61-91)], induces in rats a profound state of immobilization characterized by the absence of movement, loss of righting response, and extreme generalized muscular rigidity (*1*). Similar results have since been obtained by others (*2*). However, Jacquet and Marks (*3*) reported that injections of β-endorphin into the periaqueductal gray (PAG) elicit a "cataleptic-like" state similar to that produced by most neuroleptics (antipsychotic drugs). On the basis of these observations, they suggested that β-endorphin may be an endogenous neuroleptic and that its reduced availability may be etiologically significant in certain forms of psychopathology. Differentiation of the effects of β-endorphin as rigid immobility or neuroleptic-like catalepsy may be crucial, both with respect to mechanistic considerations as well as possible clinical implications. Therefore, we have extended our earlier studies to character-