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Carnitine Transport in Isolated Adult Rat Heart Myocytes and the Effect of 7,8-diOH Chlorpromazine

JOSEPH BAHL, THOMAS NAVIN, ALBERT A. MANIAN, AND RUBIN BRESSLER

SUMMARY We studied the carnitine transport system in isolated adult rat heart myocytes able to tolerate physiological concentrations of calcium. Carnitine uptake occurred against a concentration gradient and was inhibited by 2,4-dinitrophenol (2,4-DNP). The transport system had a Km of 60 µM and a Vmax of 110 pmol/mg protein per hour. The carnitine precursor deoxycarnitine, acetylcarnitine, and both the D and L isomers were effective inhibitors of uptake. The transport of carnitine was not dependent on sodium ions, but was stimulated by decreasing concentrations of calcium ions. Decreased uptake was observed in the presence of β-adrenergic agonists and antagonists, dibutyryl cyclic AMP, local anesthetics, and ouabain. No significant alteration of uptake was effected by atropine, carbachol or a variety of tricyclic agents. The auto-oxidation product of 7,8-dihydroxychlorpromazine (7,8-diOH CPZ) decreased carnitine efflux from myocytes, which were highly permeable to low molecular weight compounds. We found that this effect was not substrate specific, and is discussed as possibly resulting from a change in the arrangement or state of polymerization of subcellular structural components.


CARNITINE is an essential cofactor for long-chain fatty acid oxidation (Fritz, 1963). There is no cardiac synthesis of carnitine, but intracellular concentrations of carnitine are greater than plasma concentrations, suggesting an uptake system. Because of recent studies on the beneficial effects of carnitine on cardiac arrhythmias and function in the ischemic state (Vick et al., 1976; Folts et al., 1978),

The interest in carnitine transport is further increased by the observation that the rate of carnitine uptake is an index of the severity of myocardial ischemic injury (Heyndrickx et al., 1975). This work was supported by Grants HL 13636 and GM 07533.

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we undertook the characterization of an uptake system for carnitine in adult rat heart myocytes. The procedure that we used to prepare myocytes for this study yielded cells that did not undergo the rapid loss of membrane integrity as measured by trypan blue exclusion in the presence of physiological concentrations of calcium which characterized previous myocyte preparations. This provides an improved model of active cardiac myocytes (Frangakis et al., 1980).

Carnitine (γ-trimethyl-β-hydroxybutyrobetaine) is a naturally occurring compound found in high concentration in skeletal and cardiac muscle (Pearson and Tubbs, 1967). Carnitine’s role in the transfer of activated long-chain fatty acid esters of coenzyme A across the mitochondrial membrane is well known, but, in addition, a new role for carnitine has emerged from work on cardiac performance in the ischemic state. Recent studies have demonstrated beneficial effects of exogenous carnitine on animal and human cardiac functions: a vasodilating and inotropic effect in the dog (Brooks et al., 1977), protection of the myocardium from ischemic changes in the dog (Vick et al., 1976) (Folts et al., 1978), reduction of arrhythmias in the cat (DiPalma et al., 1975), protection against additional compromise of mechanical performance in ischemic swine heart caused by excess free fatty acids (FFA) (Liedtke et al., 1979), and an improved stress tolerance of the ischemic myocardium in humans (Thomsen et al., 1977).

Carnitine’s role in the metabolism of fatty acids with a reduction in intracellular levels of long-chain fatty acid esters of coenzyme A across the mitochondrial membrane is well known, but, in addition, a new role for carnitine has emerged from work on cardiac performance in the ischemic state. Recent studies have demonstrated beneficial effects of exogenous carnitine on animal and human cardiac functions: a vasodilating and inotropic effect in the dog (Brooks et al., 1977), protection of the myocardium from ischemic changes in the dog (Vick et al., 1976) (Folts et al., 1978), reduction of arrhythmias in the cat (DiPalma et al., 1975), protection against additional compromise of mechanical performance in ischemic swine heart caused by excess free fatty acids (FFA) (Liedtke et al., 1979), and an improved stress tolerance of the ischemic myocardium in humans (Thomsen et al., 1977).

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Our earlier report of energy dependent carnitine transport in myocytes (Bahl et al., 1980) employed myocytes that rapidly lost membrane integrity (trypan blue exclusion) and rapidly became maximally contracted when exposed to physiological concentrations of calcium. With the development of cardiac myocytes able to tolerate physiological concentrations of calcium, we have been able to characterize a transport system for carnitine that: (1) unlike the previous model demonstrates a high affinity transport system which conforms to Michaelis-Menten kinetics, (2) is appreciably inhibited by appropriate structural analogs, (3) has been characterized as to the effects of various ions, hormones, and cardioactive drugs. Finally, 7,8-diOH CPZ, a metabolite of chlorpromazine, has been shown to inhibit efflux of carnitine from myocytes which displayed a high degree of permeability to compounds of low molecular weight.

Methods

Adult rat heart myocytes able to tolerate physiological concentrations of calcium without rapidly losing membrane integrity and contracting into a condition of rigor were isolated according to the procedure of Frangakis et al. (1980). Hearts from adult Sprague Dawley rats (250–300 g) were perfused with Joklik MEM buffer (GIBCO) containing collagenase and hyaluronidase. Isolated cells were allowed to settle in buffer at room temperature while broken cells and non-muscle cells remained dispersed and, thus, were removed by aspiration. Approximately 80–90% of freshly prepared cells exclude the dye trypan blue and 60–75% of the cells excluded the dye after 90 minutes at 37°C in the presence of 2 mM calcium. Glycolysis, fatty acid oxidation, and the tricarboxylic acid cycle remain intact as myocytes metabolize glucose, palmitate, and pyruvate linearly for more than 2 hours. My-
ococytes not able to tolerate a physiological calcium concentration were prepared by the method of Grosso et al. (1977).

Uptake of radiolabeled carnitine was measured using $^3$H-methyl-D,L-carnitine from Amersham (1.7 Ci/mmol) and after thin layer chromatography [butanol:water:acetic acid (120:50:30)] indicated the solution was 98% homogenous and was used without subsequent purification. $^{14}$C-Sorbitol was used as a marker of extracellular space. Addition of L-(-)-carnitine (Otsuka Pharmaceutical Factory) was made to provide the carnitine concentration used for a given experiment. In a typical experiment, 2 $\mu$Ci/ml $^3$H-D,L-carnitine and 0.2 $\mu$Ci/ml $^{14}$C-sorbitol would be added to 1 ml of cells (1 mg cellular protein, approximately). The total $^3$H-carnitine measured in each aliquot of cells was corrected to yield the net $^3$H-carnitine transported into the cell. Cell water was measured using $^3$H$_2$O and similarly corrected with $^{14}$C-sorbitol. Isolated myocytes, 1 mg protein in 1.0 ml medium, were added to a plastic centrifuge tube containing labeled compounds to start the uptake process. Calcium was not added to the approximate 10 $\mu$M calcium innately contained in the incubation buffer, except as specified for a given experiment. Cells pipetted on to a 1:1 mixture of dibutyl and dioctyl phthalate esters were pelleted by centrifugation. Cells were counted after base (NaOH) hydrolysis of the pellet and subsequent neutralization (HCl). Actual dpm of $^3$H and $^{14}$C were calculated using standard double-label counting techniques (Kobayashi and Maudsley, 1974).

In those experiments where cells were filtered and rinsed rather than pelleted by centrifugation, addition of 3 ml of 1 mM unlabeled carnitine at 4°C was made to stop transport. Cells were then filtered on Nuclepore filters (10$\mu$m pore size, Nuclepore Corp.). The filters were rinsed with one additional 3-ml aliquot of 1 mM carnitine at 4°C and radioactivity on the filters measured.

L-Carnitine was measured by the method of Parvin and Pande (1977). This method measures picomole amounts of carnitine and is based on the enzymatic conversion of carnitine plus radiolabeled acetyl-CoA to radiolabeled acetylcarnitine in the presence of carnitine acetyltransferase. Protein was measured using the method of Lowry et al. (1951).

Because of the day-to-day variation in myocytes, the amount of $^3$H-carnitine transported into myocytes varied widely (5.8 to 20.5 pmol/mg protein). Values obtained on a given day were converted to percent of the matched control group mean prior to being combined with values obtained on different days. Computation of statistical significance of experimental treatment was made using the two-tailed Student's $t$-test for paired samples.

$^{14}$C-Sorbitol and other radiochemicals were purchased from Amersham. Chlorpromazine derivatives were a gift from the Psychopharmacology Research Branch of the National Institutes of Mental Health. Other chemicals were purchased from the Sigma Chemical Company.

**Results**

**Carnitine Uptake**

Total carnitine (free and carnitine esters) per mg protein was measured at the start of the perfusion with hyaluronidase and collagenase and in isolated myocytes. The isolation procedure resulted in a 69% decrease in heart myocyte carnitine concentration. Estimation of intracellular fluid volume was made by subtracting $^{14}$C-sorbitol space from total $^3$H water space found in the pellets. Using an average value for non-sorbitol space of approximately 3 $\mu$l/mg protein, an intracellular concentration of carnitine in the myocytes was calculated to be between 530 and 730 $\mu$m. Normal plasma carnitine concentration in the rat has been found to be approximately 40 $\mu$m (Marquis and Fritz, 1965, and Rebouche, 1977).

Carnitine accumulation into the non-sorbitol space was found to be biphasic, as shown in Figure 1. The initial rapid phase of carnitine uptake was not inhibited by the addition of 2,4-dinitrophenol (DNP). Iodoacetate, often used to block glycolysis, caused damage to the cell membrane making $^{14}$C-sorbitol space equivalent to $^3$H$_2$O space. We were therefore unable to establish if the initial phase of $^3$H-carnitine accumulation was dependent on energy production from glycolysis or not. The subsequent phase of carnitine accumulation was in-

![Figure 1](image-url)
hhibited strongly by DNP and represents the energy-dependent uptake of carnitine.

The effects of different ions at various concentrations on carnitine uptake were studied and the results are shown in Table 1. Uptake was not altered by substitution of LiCl for NaCl. Addition of calcium to a final concentration of 1 mM decreased carnitine uptake to 51.8% of control. Addition of 1 mM magnesium did not alter uptake in cells from matched controls.

**Effect of Carnitine Concentration**

Uptake of carnitine was measured with respect to increasing concentrations of carnitine in the incubating medium, and the results are shown in Figure 2. The transport system is composed of a high affinity saturable system which follows Michaelis-Menten kinetics and a low affinity component which does not. The low affinity system has been described previously by us (Bahl et al., 1980) and was the only system demonstrable in the preparation of myocytes that did not tolerate physiological concentrations of calcium. Using the mathematical correction of Christensen (1975) to separate the high affinity system from the low affinity system which does not show saturation, the high affinity system's $K_m$ is calculated to be 60 $\mu M$. This value is in the range of plasma carnitine concentrations. The system's $V_{max}$ was calculated to be 110 pmol/mg protein per hour. The $K_m$ measured in the isolated rat myocyte is in good agreement with the $K_m$ measured by Rebouche (1977) in the rat limb muscle, but is a somewhat lower in affinity than the 4.8 $\mu M$ measured by Bohmer et al. (1977) in cultured human heart cells.

**Effect of Structural Analogs on Uptake**

The ability of structural analogs to inhibit carnitine uptake (total high affinity and low affinity uptake) is shown in Table 2. As is the case for the carnitine transport system in rat liver, both D and L carnitine were equally effective at inhibiting uptake of $^3$H-D,L-carnitine (reduced to 17% of control). The hydroxy group on carbon 3 which establishes the D or L chirality of carnitine is not present in the metabolic precursor deoxycarnitine ($\gamma$-trimethylbutyrobetaine). Inhibition of uptake with deoxycarnitine was to 32% of control and to 27% of control with acetylcarnitine. Choline and $\gamma$-aminobutyric acid, which decreased carnitine uptake, were less effective as inhibitors. The rate of carnitine efflux from cells prelabeled with $^3$H-carnitine was not significantly increased by the presence of 40 $\mu M$ unlabeled carnitine or acetylcarnitine nor with 1 mM carnitine or acetyl carnitine, an amount far in excess of plasma levels (data not shown).

**Effect of Pharmacological Agents on Uptake**

A variety of pharmacological agents were screened for effects on carnitine uptake. As shown in Table 3, significant decreases in carnitine uptake were observed after exposure to dibutyryl cyclic AMP, the $\beta$-adrenergic agonist isoproterenol, and the antagonist propranolol. The $N,N$-dimethyl analog of propranolol which does not interact with the $\beta$-receptor as agonist or antagonist and is devoid of local anesthetic properties (Schuster et al., 1973) reduced transport to 87% of control, but failed to attain statistical significance ($P > 0.05$). The local

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**Table 1** Effect of Ions on Carnitine Uptake

<table>
<thead>
<tr>
<th>Ion</th>
<th>Control (pmol/mg protein)</th>
<th>n</th>
<th>Percent control</th>
</tr>
</thead>
<tbody>
<tr>
<td>115.5 mM LiCl/0.0 mM NaCl</td>
<td>113.0 ± 8.0</td>
<td>6</td>
<td>100 ± 7.0</td>
</tr>
<tr>
<td>1 mM Mg&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>106.8 ± 7.2</td>
<td>6</td>
<td>100 ± 3.8</td>
</tr>
<tr>
<td>0.01 mM EGTA</td>
<td>106.1 ± 3.0</td>
<td>4</td>
<td>100 ± 3.6</td>
</tr>
<tr>
<td>0.01 mM Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>117.6 ± 3.3</td>
<td>5</td>
<td>100 ± 1.9*</td>
</tr>
<tr>
<td>0.01 mM Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>150.3 ± 3.0</td>
<td>5</td>
<td>100 ± 1.9*</td>
</tr>
<tr>
<td>1.0 mM Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>51.8 ± 2.6</td>
<td>10</td>
<td>100 ± 1.9*</td>
</tr>
<tr>
<td>1.0 mM Mn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>101.8 ± 3.5</td>
<td>6</td>
<td>100 ± 3.0</td>
</tr>
</tbody>
</table>

The additional doses of substitution of ions were made for cells incubated for 1 hour with 10 $\mu M$ $^3$H-D,L-carnitine. Individual data points were converted to the percent of the mean value of the day's control before experiments from different days were combined. Values are mean ± SEM of n determinations.

* $P < 0.01$; † $P < 0.001$.

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**Table 2** Inhibition of $^3$H-D,L-Carnitine Uptake by Structural Analogs

<table>
<thead>
<tr>
<th>Structural Analog</th>
<th>Percent control</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Carnitine</td>
<td>17.5 ± 2.1</td>
<td>100 ± 3.3†</td>
</tr>
<tr>
<td>L-Carnitine</td>
<td>16.7 ± 3.2</td>
<td>100 ± 3.3†</td>
</tr>
<tr>
<td>DL-Acetylcarnitine</td>
<td>27.1 ± 3.6</td>
<td>100 ± 5.4†</td>
</tr>
<tr>
<td>Deoxycarnitine</td>
<td>32.3 ± 3.8</td>
<td>100 ± 3.1‡</td>
</tr>
<tr>
<td>Choline</td>
<td>88.7 ± 2.2</td>
<td>100 ± 2.5*</td>
</tr>
<tr>
<td>GABA</td>
<td>73.3 ± 4.6</td>
<td>100 ± 2.7‡</td>
</tr>
</tbody>
</table>

Uptake of 10 $\mu M$ $^3$H-D,L-carnitine for 1 hour was measured in the presence and absence of the structural analogs (1 mM) listed above. Values expressed as percent of control are the mean ± SEM (n = 8).

* $P < 0.02$; † $P < 0.01$; ‡ $P < 0.001$. 

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**Table 3** Inhibition of $^3$H-D,L-Carnitine Uptake by Pharmacological Agents

<table>
<thead>
<tr>
<th>Pharmacological Agent</th>
<th>Percent control</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibutyryl cyclic AMP</td>
<td>16.7 ± 2.6</td>
<td>100 ± 3.8</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>15.3 ± 3.0</td>
<td>100 ± 1.9*</td>
</tr>
<tr>
<td>Propranolol</td>
<td>45.8 ± 2.6</td>
<td>100 ± 3.8</td>
</tr>
</tbody>
</table>

Uptake of 10 $\mu M$ $^3$H-D,L-carnitine for 1 hour was measured in the presence and absence of the pharmacological agents (1 mM) listed above. Values expressed as percent of control are the mean ± SEM (n = 8).
TABLE 3  Effect of Pharmacological Agents on Carnitine Transport

<table>
<thead>
<tr>
<th>Drug, 10^{-5} M</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>90.7 ± 2.7*</td>
</tr>
<tr>
<td>Dibutyl cyclic AMP</td>
<td>81.7 ± 3.8§</td>
</tr>
<tr>
<td>Propranolol</td>
<td>74.3 ± 5.5*</td>
</tr>
<tr>
<td>UM 272 (N,N-dimethyl-propranolol)</td>
<td>87.1 ± 7.5</td>
</tr>
<tr>
<td>Verapamil</td>
<td>76.4 ± 4.4</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>76.0 ± 1.4</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>76.3 ± 2.5</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>81.7 ± 4.1§</td>
</tr>
</tbody>
</table>

Effects of pharmacological agents on ³H-carnitine uptake. Myocyte uptake of 50 μM ³H-carnitine was measured after 1 hour at 37°C in the presence of various agents at 10^{-5} M. Values expressed as mean ± SEM of control uptake. Ouabain studies were done at the concentration, with or without calcium, as indicated by the table.

* P < 0.05; § P < 0.02; §§ P < 0.01; §§§ P < 0.001.

TABLE 4  Effect of 7,8-diOH CPZ on ³H-Labeled Carnitine, Deoxyglucose, and α-Amino Isobutyric Acid

<table>
<thead>
<tr>
<th>Method</th>
<th>7,8-diOH CPZ</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>³H-Carnitine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous cells</td>
<td>F</td>
<td>353.0 ± 18.2</td>
</tr>
<tr>
<td>Ca²⁺ tolerant cells</td>
<td>F</td>
<td>152.3 ± 10.7</td>
</tr>
<tr>
<td>Ca²⁺ tolerant cells</td>
<td>P</td>
<td>105.0 ± 2.0</td>
</tr>
<tr>
<td>³H-Deoxyglucose</td>
<td>F</td>
<td>116.0 ± 3.5</td>
</tr>
<tr>
<td>³H-α-Aminoisobutyric acid</td>
<td>F</td>
<td>123.0 ± 5.0</td>
</tr>
</tbody>
</table>

Cells were either filtered (F) or pelleted (P) as described in Methods. Incubations were with or without 10^{-5} M 7,8-diOH CPZ for 10 minutes. Previous cells refers to myocytes not able to tolerate physiological calcium, prepared by the method of Gruosso et al. (1977). All other cells were prepared by the method of Frangakis et al. (1980). Values are the mean ± SEM (n = 8).

* P < 0.05; § P < 0.02; † P < 0.01; ‡ P < 0.001.

Previous cells refers to myocytes not able to tolerate physiological calcium, prepared by the method of Gruosso et al. (1977). All other cells were prepared by the method of Frangakis et al. (1980). Values are the mean ± SEM (n = 8).

* P < 0.05; † P < 0.01; ‡ P < 0.001.

anesthetics, lidocaine and tetracaine, however, were equally effective in decreasing carnitine transport.

Ouabain decreased transport of carnitine at concentrations above 10^{-5} M in myocytes with no added calcium, but only at 10^{-4} M if 1 mM calcium was present. No significant alteration in carnitine transport was observed in myocytes treated with verapamil, which acts at the external surface of the cell to block slow-channel ion movement such as calcium (Shigenobu et al., 1974), nor was any effect observed with chlorpromazine. Similarly, no significant alteration of carnitine transport was observed with thioridazine, haloperidol, stelazine, doxepine, imipramine, chlorimipramine, atropine, or carbachol (data not shown).

In our earlier studies of carnitine transport, we reported (Bahl et al., 1980) that the auto-oxidation product of 7,8-diOH CPZ produced an energy dependent 2- to 4-fold increase in carnitine accumulation. This increase was correlated with the magnitude of the absorbance peak at 510 nm from the product of auto-oxidation of 7,8-diOH CPZ, and only structural analogs readily undergoing auto-oxidation to a phenothiazine ortho-quinone were found capable of effecting this increase in heart myocyte ³H-DL-carnitine accumulation. Maximal differences were observed after 10-15 minutes of phenothiazine exposure and increasing concentrations of calcium progressively diminished the magnitude of the effect. The data in Table 4 show that when myocytes treated with 7,8-diOH CPZ were filtered our previous (more permeable) heart cell preparations and our current cell preparation showed quantitative differences in carnitine accumulation. The more permeable cells with 7,8-diOH CPZ showed 3.5 times the accumulation of ³H-carnitine than did the matched controls, whereas the current preparation of myocytes had only 1.5 times the accumulation of ³H-carnitine above the matched controls. When cells were centrifuged through a non-aqueous medium into a pellet, rather than being filtered and rinsed, no stimulation of carnitine accumulation was observed. No aggregation of cells was observed in the presence or absence of 7,8-diOH CPZ. Similar values were observed with 7,8-dioxo CPZ, the quinone form of the dihydroxy compound (data not shown). To determine the specificity of the 7,8-diOH CPZ effect as measured by filtration method, ³H-2-deoxyglucose (2-DG) and ³H-α-aminoisobutyric acid (AIB) accumulation was measured. Myocytes incubated with 7,8-diOH CPZ for 10 minutes had greater radioactivity than control cells for both 2-DG (116% of control) and AIB (112% of control) (Table 4). The stimulation proved to be a transient one, with incubations beyond 30 minutes not showing significant increases in accumulation of these compounds, nor did introduction of the drug to cells for a second exposure provide additional stimulation, suggesting that the cell system was capable of being stimulated only the one time (data not shown).

By preloading cells with ³H-carnitine and using the centrifugation technique, it was possible to show that 7,8-diOH CPZ had an effect on efflux (Fig. 3). Myocytes treated with 7,8-diOH CPZ after preloading with ³H-carnitine displayed lower carnitine efflux at the time points studied (P < 0.05). The efflux curve is biphasic with the initial phase of efflux being rapid and more sensitive to the presence of 7,8-diOH CPZ than the second, slower, phase of efflux. This suggested that 7,8-diOH CPZ might be decreasing permeability.

Because 2-DG, carnitine, and sorbitol demonstrated nearly identical efflux curves during the initial rapid phase, it seems probable that the initial rapid phase represents efflux of label from cells which are highly permeable to labels of this sort,
Carnitine transfer of fatty acids all are stereoselective for L-carnitine. Because only the L-isomer is produced in vivo, the lack of stereoselectivity by the transport system would not in any way limit its normal utility to the cell. Inclusion of acetylcarnitine or carnitine during 3H-carnitine efflux from preloaded cells did not provide evidence for countertransport of carnitine out of the cell.

Regulation of the carnitine transport system appears complex, but sensitive to calcium. Addition of 1 mM calcium decreased carnitine uptake by 50%. Yet, manganese which enters the cell and often acts as a calcium congener, did not decrease uptake, nor did the blocker of the slow channel, verapamil. Ouabain decreased carnitine uptake significantly at concentrations above 10^{-6} M when no calcium was added to the incubation medium, but only at 10^{-4} M when 1 mM calcium was added.

The β-adrenergic agonist, isoproterenol, and the antagonist, propranolol, decrease carnitine uptake by comparable amounts. The local anesthetics, lidocaine and tetracaine, were effective inhibitors of carnitine transport. The cholinergic agonist, carbachol, and the antagonist, atropine, failed to alter carnitine transport, as did a variety of tricyclic agents. These data suggest that control of carnitine transport in vivo may be sensitive to a variety of endogenous compounds and xenobiotics, although the regulatory controls are not yet discernible.
In our initial studies (Bahl et al., 1980), myocytes that could not tolerate physiological levels of calcium were exposed to 7,8-diOH CPZ, filtered, and rinsed with buffer. These cells contained 2 to 4 times more $^3$H-carnitine than their matched controls. This effect was inhibited by 2,4-DNP and diminished by calcium. Myocytes that could tolerate physiological concentrations of calcium showed a similar, albeit lesser effect when measured by this method (Table 2). No effect of 7,8-diOH CPZ on carnitine uptake by the same cells could be found when the centrifugation technique was used. Thus, 7,8-diOH CPZ was influencing net accumulation of carnitine as measured by filtration, but was not altering net uptake into cell space corrected for $^3$H-carnitine in the $^{14}$C-sorbitol space of the pellet as measured by centrifugation. The disparity between filtration and centrifugation methods was repeated with $^3$H-2-DG and $^3$H-AIB and suggests that the effect is not substrate specific. Efflux studies suggested 7,8-diOH CPZ had changed the amount of carnitine lost from highly permeable cells. Less trypan blue entered cells after treatment with 7,8-diOH CPZ, suggesting a physical change had occurred in at least some of the cells which otherwise would have been found to be permeable to the dye.

The disparity between the filtration and centrifugation technique thus appears to result from different quantities being measured. The filtration technique measured material remaining after rinsing. An agent that helped to seal permeable cells would result in more carnitine remaining on the filter. The centrifugation technique measured only carnitine in nonsorbitol space. Because labeled sorbitol and carnitine would rapidly enter highly permeable cells in a constant ratio, subsequent changes in membrane permeability would not be detected with the centrifugation method. The large variation in the 7,8-diOH effect may relate not only to the extent of auto-oxidation of 7,8-diOH CPZ, but also to the extent of membrane permeability. The cells which were not able to tolerate physiological calcium displayed greater membrane permeability than cells able to tolerate physiological calcium (Frangakis et al., 1980) and showed a greater 7,8-diOH CPZ effect on carnitine remaining on filters.

A possible explanation for the mechanism of 7,8-diOH CPZ action may be provided by the work of Elias and Boyer (1979). They showed that certain hydroxylated chlorpromazine metabolites, including 7,8-diOH CPZ, possessed the ability to increase the extent of actin polymerized in vitro and suggested that the in vivo sol-gel state of a cell might be altered by this class of compounds. It is, therefore, suggested that cells which might otherwise leak their contents through limited openings in the membrane may, by proper arrangement and degree of polymerization of internal and/or membrane structural components, be able to limit movement through these openings.

Increasing calcium concentration decreased the effect observed with 7,8-diOH CPZ (Bahl et al., 1980). Chelation of calcium by 7,8-diOH CPZ decreases the autooxidation to the quinone 7,8-diOH CPZ (Rajan et al., 1974) and may reduce the availability of the active drug. Calcium is known to catalyze the depolymerization of many intracellular polymers (Lazarides, 1980). It is interesting to note that low temperatures, which result in depolymerization of certain structural components in cells, had to be avoided in the preparation of myocytes that could tolerate physiological concentrations of calcium. The action of 7,8-diOH CPZ may provide a useful biochemical tool in studying how impaired cells maintain their integrity. The pharmacological utility of this agent may be limited by high levels of plasma calcium and by the relatively rapid auto-oxidation or clearance of the chlorpromazine metabolite.

The 7,8-diOH metabolite of CPZ is unusually cardioactive (Akera et al., 1974). This metabolite has been reported to produce a positive inotropic effect in isolated guinea pig hearts (Akera et al., 1977), causes release of catecholamines (Temma et al., 1977), and inhibits Na$^+$/$K^+$-ATPase activity (Akera et al., 1977). Recently, administration of chlorpromazine was reported to prevent in vivo acceleration of phospholipid degradation and associated membrane dysfunction in ischemic myocardial cells (Chien et al., 1979). This effect may, at least in part, be due to a metabolite of CPZ, but the mechanism which might unite this effect and the ones observed for 7,8-diOH CPZ remains to be studied.

We have characterized the carnitine transport system in isolated rat heart myocytes with the belief that this information would be of value in future work on the role of carnitine in myocardial ischemia and its treatment. The effect of 7,8-diOH CPZ also was investigated. The data suggest that, rather than specifically affecting carnitine transport, the cell membrane permeability decreases after treatment with 7,8-diOH CPZ. The potential of 7,8-diOH CPZ to decrease the efflux of carnitine that occurs during ischemia remains to be studied. This agent may also be useful in studying the ability of damaged cells to maintain homeostasis.

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