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Furosemide and digoxin inhibit thiamine uptake in cardiac cells

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Abstract

Heart cells in culture were used to clarify whether furosemide or digoxin cause thiamine deficiency and if so, by what mechanism. The intracellular level of thiamine pyrophosphate gradually decreased, with a half-life of 16–19 days, after treatment of cardiac cells with furosemide or digoxin. When thiamine was excluded from the growth medium, thiamine pyrophosphate levels gradually decreased, with a half-life of 5–6 days. No additive effect was observed in the presence of the above drugs when thiamine was excluded from the medium. Thiamine uptake by cardiac cells grown in a thiamine-free medium for 7 days decreased significantly in the presence of furosemide or digoxin. The effect of furosemide or digoxin on thiamine uptake was found to be dose dependent. Co-administration of furosemide and digoxin to the cardiac cell cultures resulted in an additive effect on thiamine uptake. Our results demonstrate that furosemide and digoxin inhibit thiamine uptake by cardiac cells in culture and may therefore cause thiamine deficiency in patients undergoing chronic treatment with these drugs.

Keywords: Thiamine; Cardiac cell; Furosemide; Digoxin

1. Introduction

Thiamine (vitamin B1) deficiency may cause damage to the nervous system ('dry beriberi') and/or the cardiovascular system ('wet beriberi'). After uptake, thiamine undergoes double phosphorylation to yield thiamine pyrophosphate. Thiamine pyrophosphate is an important cofactor in several vital enzymatic reactions which are involved in metabolism and energy production. Thiamine uptake by the cell is affected by changes in the sodium gradient and, in some cases, is even dependent on it (Ferrari et al., 1971; Lumeng et al., 1979).

It has recently been claimed, based on thiamine pyrophosphate effect measurements in red blood cells, that chronic use of diuretic drugs can cause thiamine deficiency (Yui et al., 1980; Seligmann et al., 1991; Shimon et al., 1995). In rat small intestine membrane vesicles, [^{3}H]thiamine uptake decreased by 30% in the presence of 1.5 mM furosemide and by 35% in the presence of 2 mM ouabain (Laforenza et al., 1993). Ouabain treatment also resulted in a decrease in thiamine uptake in isolated hepatocytes (Lumeng et al., 1979). The effect of furosemide or ouabain on thiamine uptake by other tissues has not been reported to date.

Since furosemide and digoxin are used in the treatment of cardiac patients, and since thiamine deficiency may cause further impairment of myocardial performance (McIntry and Stanley, 1971; Cappelli et al., 1990; Zangen and Shainberg, 1997), we examined whether these drugs may affect thiamine pyrophosphate levels or thiamine uptake in rat cardiac cells in culture.

2. Methods

2.1. Cell culture

Rat (1–2 days old) hearts were removed under sterile conditions and washed three times in phosphate buffered saline (PBS) to remove excess blood cells. The hearts were minced into small fragments and then agitated gently in a solution of proteolytic enzyme-'RDB' (Ness-Ziona, Israel) prepared from a fig tree extract. The proteolytic enzyme was diluted 1:50 in Ca^{2+}- and Mg^{2+}-free PBS, at 25°C for several cycles of 10 min each, as described previously (Brik and Shainberg, 1990; Weinstein et al., 1991). Dulbecco's modified Eagle's medium (DMEM) containing

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10% horse serum (Biological Industries, Kibbutz Beit Haemek, Israel) was added to supernatant suspensions containing dissociated cells. The mixture was centrifuged at 150 g for 5 min, the supernatant phase was discarded, and the cells were resuspended. The cell suspension was diluted to 1.0×10^6 cells/ml and 1.5 ml were placed in 35 mm collagen-gelatin-coated plastic culture dishes. The cultures were incubated in a humidified atmosphere of 5% CO_2, 95% air at 37°C. Confluent monolayers, which exhibited spontaneous contractions, developed in culture within 2 days. The growth medium was replaced after 24 h and then every 3 days. In some cultures the growth medium was replaced by a special 'thiamine-free' medium based on DMEM without thiamine, supplemented with 10% horse serum. All cell cultures contracted spontaneously and looked normal at the day of measurements, including the drug treated cultures.

2.2. Determination of intracellular thiamine pyrophosphate

Analysis of thiamine pyrophosphate was carried out by high-performance liquid chromatography (HPLC) (Waters M-510), using the method described by Tallaksen et al. (1991), with minor modifications for adjusting the assay to cardiac cultures, as described previously (Zangen and Shainberg, 1997). The cardiac cells were washed with PBS, harvested with a rubber policeman under 1 ml 1% triton × 100 solution, and homogenized for 10 s at 4°C. Deproteinization was performed by the addition of 70 μl 50% trichloroacetic acid solution to 0.7 ml of the cell homogenate. After 10 min of centrifugation at 2000 g, the supernatant phase was transferred to fresh glass tubes, and the trichloroacetic acid was extracted twice with five volumes of water-saturated diethyl ether. The thiamine pyrophosphate was derivatized by the addition of 60 μl 0.3 M cyanogen bromide solution to 0.6 ml of the sample. A 100 μl aliquot of the derivatization sample was injected into the HPLC. A Merck Licrosorb NH_2 column (15 mm × 4.6 mm i.d.) was used. The mobile phase consisted of acetonitrile and phosphate buffer (85 mM, pH 7.5) 50:50 (v/v). The flow-rate was 1.5 ml/min, and the thiamine pyrophosphate retention time was 5.6 min. The detection limit was 4 pg on the column, and the intra-assay variation coefficient, calculated on the basis of ten analyses of the same sample on one day, was 6.2%.

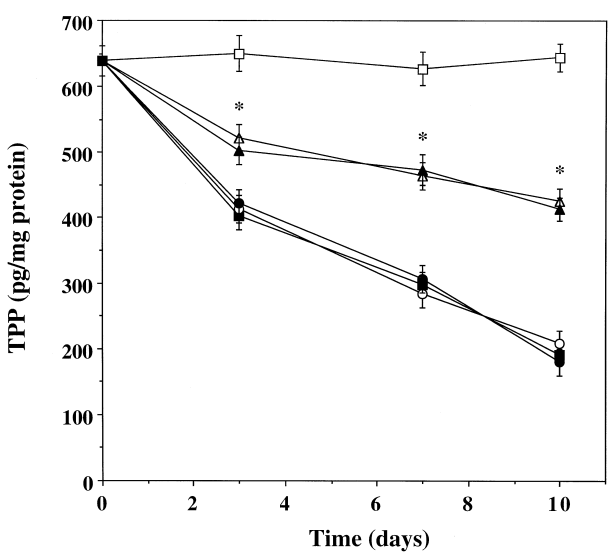

Fig. 1. Cardiac cells were grown in normal medium for 3 days after which furosemide 5 μg/ml (△), or 25 μg/ml (▲) was added to the medium. Control cells were not treated with any drug (□). In another group the medium was replaced by 'thiamine free' medium (■). (○) 'Thiamine free' medium with 5 μg/ml furosemide. (●) 'Thiamine free' medium with 25 μg/ml furosemide. Thiamine pyrophosphate levels were measured after 3, 7 and 10 days. $* P < 0.05$ ($n = 5$).

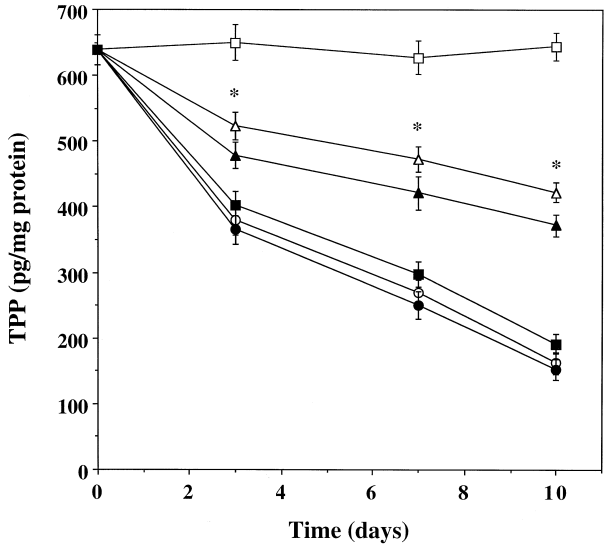

Fig. 2. Cardiac cells were grown in normal medium for 3 days, after which digoxin 10 ng/ml (△), or 50 ng/ml (▲) was added to the medium. Control cells remained in the same medium (□). In another group the medium was replaced by 'thiamine free' medium (■). (○) 'Thiamine free' medium with 10 ng/ml digoxin. (●) 'Thiamine free' medium with 50 ng/ml digoxin. Thiamine pyrophosphate levels were measured after 3, 7 and 10 days in each group. $* P < 0.05$ ($n = 5$).

2.3. Determination of [^{14}C]thiamine uptake by cardiac cells

[^{14}C]Thiamine (24 mCi/mmol) was purchased from Amersham. For determination of thiamine uptake, cardiac cells received 0.025 μCi/ml [^{14}C]thiamine (1 μM) for 30 min at 37°C. The cardiac cells were then washed five times with cold PBS, harvested with a rubber policeman and radioactivity was determined. For non-specific uptake, 100 μM unlabeled thiamine was included together with labeled thiamine. The non-specific counts (373 ± 56 DPM) were subtracted from the total uptake (966 ± 274 DPM for control cultures) in all samples. Data are presented as mean ± S.E.M., as percent of control.

2.4. Protein determination

Protein content was determined according to the method of Lowry et al. (1951), using bovine serum albumin as standard.

2.5. Statistics

The significance of the differences of the means for different experimental conditions was evaluated using the Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of furosemide and digoxin on thiamine pyrophosphate levels in normal cardiac cells

Cardiac cells, grown in normal medium for 3 days, received furosemide (5 μg/ml or 25 μg/ml) or digoxin (10 ng/ml or 50 ng/ml) in order to study the effects of sodium transport inhibitors on the thiamine pyrophosphate level. Control cells were not treated with any drug. In another group, the medium was replaced by a thiamine-free medium containing furosemide or digoxin at the same concentrations as described above. The control for the latter group of cardiac cells was treated with thiamine-free medium without any drug. Thiamine pyrophosphate levels were measured after 3, 7, and 10 days.

Both drugs resulted in a growing decrease in thiamine pyrophosphate levels during the experimental period. Furosemide caused a significant 19.2% or 22.9% decrease after 3 days and a significant 34.6% or 36.7% decrease after 10 days (for the lower and higher dose of furosemide, respectively), without significant differences between the 2 doses of furosemide (Fig. 1). Digoxin caused a significant 19.3% or 26.9% decrease after 3 days and a significant 32.9% or 40.7% decrease after 10 days (for the lower and higher dose of digoxin, respectively), with a significant difference between the 2 doses of digoxin (Fig. 2).

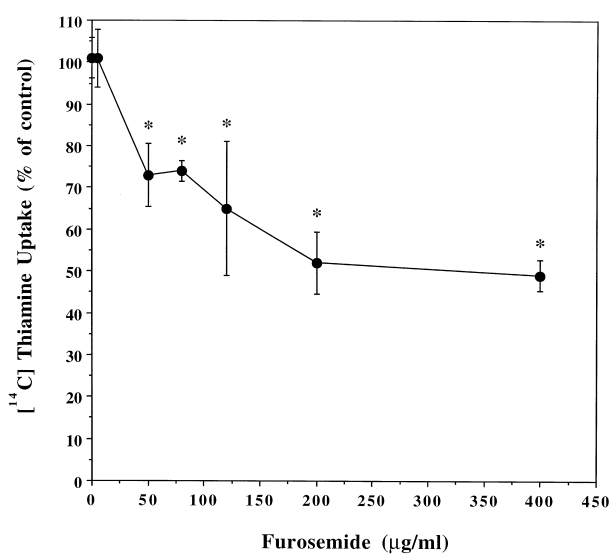

Fig. 3. Cardiac cells were grown in a thiamine-free medium for 5 days. On the 5th day the cells received various concentrations of furosemide for 48 h. They were then pulsed with [^{14}C]thiamine for 30 min at 37°C. Non-specific counts were subtracted from each sample. Results are presented as percentage of control (mean ± S.E.M.). $* P < 0.05$ ($n = 5$).

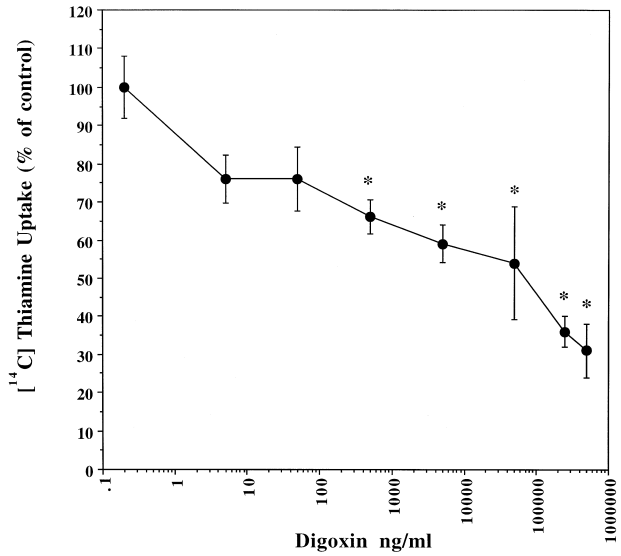

Fig. 4. Cardiac cells were grown in a thiamine-free medium for 5 days. On the 5th day the cells received various concentrations of digoxin for 48 h. They were then pulsed with [^{14}C]thiamine for 30 min at 37°C. Non-specific counts were subtracted from each sample. Results are presented as percentage of control (mean ± S.E.M.). $* P < 0.05$ ($n = 5$).

When the cells were treated with a thiamine-free medium, no significant differences were observed between the thiamine pyrophosphate levels of furosemide- or digoxin-treated cells and the controls. The rate of thiamine pyrophosphate elimination was similar in all cases, with a half-life of 5–6 days. Digoxin seemed to cause a greater decrease in thiamine pyrophosphate levels but this difference was not significant (Figs. 1 and 2).

3.2. Effect of furosemide and digoxin on thiamine uptake

Cardiac cells were grown for 5 days in a thiamine-free medium, in order to clarify the mechanism by which these drugs exert their effect on thiamine pyrophosphate levels. Furosemide (5, 50, 80, 120, 200, 400 μg/ml) or digoxin (5, 50, 500 ng/ml or 5, 50, 250, 500 μg/ml) were then added to the medium. Specific [^{14}C]thiamine uptake was measured after 2 days. The control cells were grown in the same medium for 7 days, and were not treated with any drug. The results demonstrate a dose-dependent decrease in thiamine uptake in the presence of furosemide (from a 28% decrease at 50 μg/ml to a 62% decrease at 400 μg/ml furosemide) or digoxin (from a 24% decrease at 50 ng/ml to a 66% decrease at 500 μg/ml digoxin) compared to the control cells (Figs. 3 and 4).

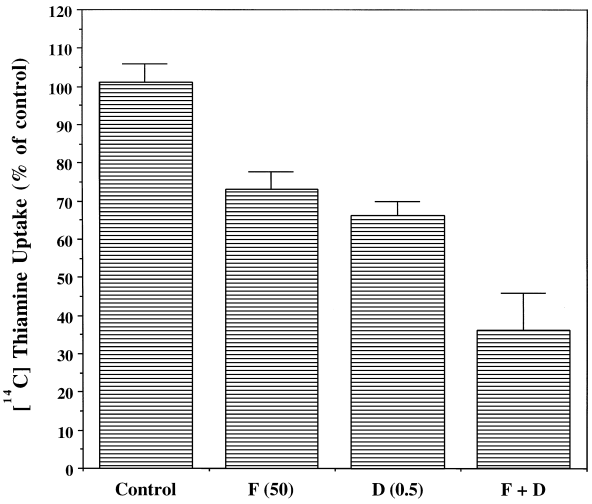

Fig. 5. Cardiac cells were grown in a thiamine-free medium for 5 days. On the 5th day the cells received 50 μg/ml furosemide (F 50) or 0.5 μg/ml of digoxin (D 0.5) or both (F+D) for 48 h. They were then pulsed with [^{14}C]thiamine for 30 min at 37°C. Non-specific counts were subtracted from each sample. Results are presented as percentage of control (mean ± S.E.M.).

3.3. Effect of co-administration of furosemide and digoxin on thiamine uptake

An additive effect was observed when the cardiac cells were treated concomitantly with furosemide and digoxin. The results for co-administration of 50 μg/ml furosemide and 0.5 μg/ml digoxin are presented in Fig. 5. The total reduction in thiamine uptake (64.1%) was equivalent to the sum of the effects of 50 μg/ml furosemide alone (27.3%) and 0.5 μg/ml digoxin alone (34.8%).

4. Discussion

This study was undertaken in order to determine whether furosemide or digoxin may affect thiamine uptake or thiamine pyrophosphate levels in rat cardiac cells in culture. The results of this study indicate that furosemide and digoxin indeed inhibit thiamine uptake in cardiac cells and may therefore cause thiamine deficiency in patients undergoing chronic treatment with these drugs.

The reason for thiamine pyrophosphate reduction during furosemide or digoxin treatment is not due to an increase in thiamine pyrophosphate utilization, but to a decrease in thiamine pyrophosphate production. Thiamine pyrophosphate levels in the cardiac cells gradually decreased in the presence of furosemide and digoxin, but in thiamine-free medium no significant differences were observed in cells exposed to furosemide or digoxin, compared with untreated cells (Figs. 1 and 2). The decrease in thiamine pyrophosphate production may be caused by inhibition of either thiamine uptake or the phosphorylation processes. Laforenza et al. (1993), who examined [^{3}H]thiamine uptake directly using membrane vesicles from rat small intestine, observed a 30% decrease in the presence of 1.5 mM furosemide and a 35% decrease in the presence of 2 mM ouabain. We used lower concentrations of furosemide and digoxin, which are similar to the therapeutic concentrations found in human plasma (5 μg/ml furosemide and 10 ng/ml digoxin). Significant reductions of thiamine pyrophosphate levels in cardiac cells were found, although the effective therapeutic doses of digoxin are different between humans and rats (Charlemagne, 1993). However, when [^{14}C]thiamine uptake was measured directly, 50 μg/ml furosemide or 0.5 μg/ml digoxin were required for a significant effect. Lower doses of furosemide and digoxin did not have a significant effect. This may be due to the short [^{14}C]thiamine uptake procedure (only 30 min), while measurements of changes in thiamine pyrophosphate levels were performed after at least 3 days. The high doses of digoxin necessary to inhibit thiamine uptake may reflect the uniqueness of rat heart which contain α1, α2 and α3 isoforms of Na^+/K^+-ATPase (Na^+ pump) which have vastly different ouabain affinities in rat but not in human (O'Brien et al., 1994). In fact the dose response curve (Fig. 4) which covers several order of magnitude, is consistent with the idea that all α isoforms (low and high affinity for digitalis) are expressed in the cultured rat myocytes, whereas in humans the therapeutic range for cardiac glycoside treatment is very narrow, only 1–2 ng digoxin/ml (Marcus et al., 1991).

The additive effect of furosemide and digoxin on the inhibition of [^{14}C]thiamine uptake (Fig. 5) indicates that their inhibitory mechanism is not contradictory, and may even be complementary. It therefore seems unlikely that thiamine uptake is dependent on the intracellular Ca^{2+} concentration, since furosemide and digoxin should have an antagonistic effect on intracellular Ca^{2+} (Rubin et al., 1995). Thiamine uptake inhibition may be associated with the effects of furosemide and digoxin on the sodium gradient: digoxin, like ouabain, inhibits the Na^+/K^+ ATPase, leading to elevation of intracellular sodium levels and thus to a reduction in the sodium gradient (Smith, 1988). However, furosemide inhibits the $Na^+/K^+/Cl^-$ co-transport (Chipperfield, 1986; Frelin et al., 1986), leading to an increase in the sodium gradient. A possible explanation may be that the $Na^+/K^+/Cl^-$ co-transport function is a prerequisite for thiamine uptake. The effect of furosemide would therefore be understandable. However, when the sodium gradient is decreased (by substances such as digoxin), $Na^+/K^+/Cl^-$ co-transport is also inhibited, since the sodium gradient is a driving force for this passive co-transport (Liu et al., 1989).

The thiamine pyrophosphate concentrations after 10 days exposure to furosemide or digoxin did not fall below 400 pg/mg protein. In a previous study we demonstrated that impaired ATP production and reduced contraction amplitude occur only when protein concentration falls below 100 pg/mg (Zangen and Shainberg, 1997). How-

ever, the data show that chronic use of these drugs may cause thiamine deficiency and therefore direct damage to cardiac muscle.

Recent clinical research has reported improved left ventricular function after thiamine supplementation in patients undergoing long-term furosemide therapy (Shimon et al., 1995). As far as we know, no such clinical research has yet been conducted on digoxin.

In conclusion, our data, together with the clinical data, point to the need for complementary thiamine treatment for patients undergoing chronic treatment with furosemide or digoxin.

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