EFFECT OF STRESS HORMONES ON THE ^{59}Fe TRANSFERRIN BINDING TO CELL MEMBRANES FROM RAT KIDNEY, BRAIN AND HEART

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ABSTRACT

The aim of the present study was to estimate *in vitro* effect of adrenaline, noradrenaline, L-DOPA and cortisol (10^{-6}M) on the specific binding of ^{59}Fe transferrin (^{59}FeTf) to cell membranes from rat heart, brain and kidney. Adrenaline lowered reliably ^{59}FeTf binding to cell membranes of heart, but L-DOPA has the opposite effect. Noradrenaline inhibited binding of ^{59}FeTf to brain cell membranes (by 33%, $p<0.001$). Cortisol enhanced reliably ^{59}FeTf binding to the cell membranes of all tested organs, the effect being most pronounced in cell membranes, isolated from the brain (by 92%, $p <0.001$). The results obtained would be useful in the study of changes in iron homeostasis in patological conditions with high content of catecholamines and cortisol.

Key words: ^{59}Fe transferrin, membranes, hormones, stress, receptors

Introduction

The body reacts to stress first by releasing the catecholamine hormones, adrenaline and noradrenaline, and the glucocorticoid hormones, cortisol and cortisone (Benedict et al. 1978). Plasma adrenaline and noradrenaline concentrations and dopamine-beta-hydroxylase activities were measured in patients with septicaemic, traumatic or haemorrhagic shock. Irrespective of the type of shock plasma adrenaline and noradrenaline concentrations were increased above the normal range **(**Benedict et al. 1978). Cardiopulmonary bypass is associated with substantial release of catecholamines and cortisol for 12 or more hours (Plunkett et al. 1997).

Various studies have suggested that the ability to generate reactive oxygen species by redox cycling quinones and catecholamines may require metal ions. (Stohs and Bagchi, 1995) DOPA also may play an important role in the maintenance of transition-metal homeostasis as an iron chelator (Nappi and Vass 2000). Adrenaline was shown to form colored complexes with iron (Ryan et al. 1993). Some microorganisms sequester iron from complexes with catheholamines: Noradrenaline-Tf complexes, but not noradrenaline - apo-Tf complexes stimulated bacterial growth. Incubation with concentrations of noradrenaline that stimulated bacterial growth resulted in loss of bound iron from iron-saturated Tf, as indicated by the appearance of monoferric- and apo-isoforms upon electrophoresis in denaturating gel (Freestone et al. 2000). Some data demonstrated that dopamine and norepinephrine can function as siderophore-like compounds in *L. monocytogenes* owing to their ortho-diphenol function and that catecholamine-mediated iron acquisition does not involve specific catecholamine receptors but acts through a cell-bound ferrireductase activity (Coulanges et al. 1998). In literature no data on how such complexes of catecholamines involved in iron transport and absorption from other cell types.

The aim of the present study was to estimate *in vitro* effect of the hormones – mediators of stress (adrenaline, noradrenaline, DOPA and cortisol) on the specific binding of ^{59}FeTf to cell membranes from rat heart, brain and kidney.

Material and Methods

Adrenaline, noradrenaline, DOPA and cortisol were supplied from Sigma, St.Louis, MO, USA.

White Wistar rats one month age were used. Tissues were homogenized in PBS (pH 7.4) at 4^{o}C. After centrifugation at 600g, 4^{o}C, 30 min, was obtained the nuclear fraction that is discarded. Then supernatatnt was centrifugated at 20000 g at 4^{o}C for 30 min (Frantz et al. 1974). The isolated cell membranes were washed three times under the same condition. Membrane protein concentration was measured by Lowry et al. (1951).

Apo-Tf was prepared by dialyzing Tf against 0.1 M acetate buffer, pH 4.5, at room temperature for 24 h with two changes of distillated water. A volume of 5 ml of apo-Tf (8mg/ml) was mixed with 0.5 ml of 0.5M bicarbonate and 2.5 ml ^{59}Fe ascorbinate (containing 6.8 x 10^{-7}g Fe with radioactivity 9.25 MBq). This mixture was incubated at 37^{o}C for 48 h. Finally, the unbound ^{59}Fe was removed by dialyzing the preparation against 20mM $NaHCO_3$, pH 8.8, at room temperature for 24h. The purity of ^{59}FeTf was tested by SDS-PAGE.

Incubation mixture for estimation of ^{59}FeTf binding contained: 100 µl cell membranes (1µg protein), 100 µl ^{59}Fe Tf (10^{-6} mol/l with specific activity 0.4 KBq/mg), 100 µl non-labelled Tf (100 µg), 100 µl of tested hormones (10^{-6}M) and 100 µl PBS (pH 7.4). In the samples for measuring the total binding non-labelled Tf was not added. Specific binding was calculated by subtracting nonspecific binding value from the total binding value. The incubation time was 30 min. Reaction was stopped by the addition of 1 ml ice-cold PBS (ph 7.4) and cell membranes collected by centrifugation at 6000 x g at 4^{o}C for 20 min. The radioactivity was measured in a Rack Gamma - 1270 counter (LKB-Wallac, Turku, Finland). The results are presented as specifically bound ^{59}FeTf, expressed as g Fe x 10^{-9} (Angelova-Gateva 1980).

Results

Brain - Noradrenaline and cortisol exhibit opposite effects on the binding of 59FeTf with cell membranes of the brain. Noradrenaline inhibited by 33% ($p < 0.001$) 59FeTf binding to cell membranes of brain. Cortisol enhances the binding of ^{59}FeTf to cell membranes of the brain (by 92%, $p < 0.001$); Adrenaline and L-DOPA have no reliable effect (Table 1 and Figure 1)).

Kidney - Only cortisol reliably causes an increase in ^{59}FeTf binding with cell membranes of kidney (38%, $p < 0.001$). The other hormones tested do not have a reliable effect (Table 1 and Figure 1).

Heart - Cortisol and L-DOPA stimulated the binding of ^{59}FeTf with cell membranes of heart, respectively. with 43% ($p < 0.001$) and 31% ($p < 0.001$). Adrenaline decreased reliably ^{59}FeTf binding to cell membranes isolated from rat hearts (with 39%, $p < 0.001$), (Table 1 and Figure 1).

Adrenaline reduces reliably ^{59}FeTf binding to the cell membranes of heart. On the other organs have not reliable effect. (Table 1 and Figure1).

L-DOPA increases 59FeTf binding with the cell membranes of heart reliably. On the other organs have not reliable effect. (Table 1 and Figure1).

Noradrenaline - Noradrenaline inhibits the binding of ^{59}FeTf to cell membranes of the brain, but it have no reliable effect on the other organs (Table 1 and Figure 1). .

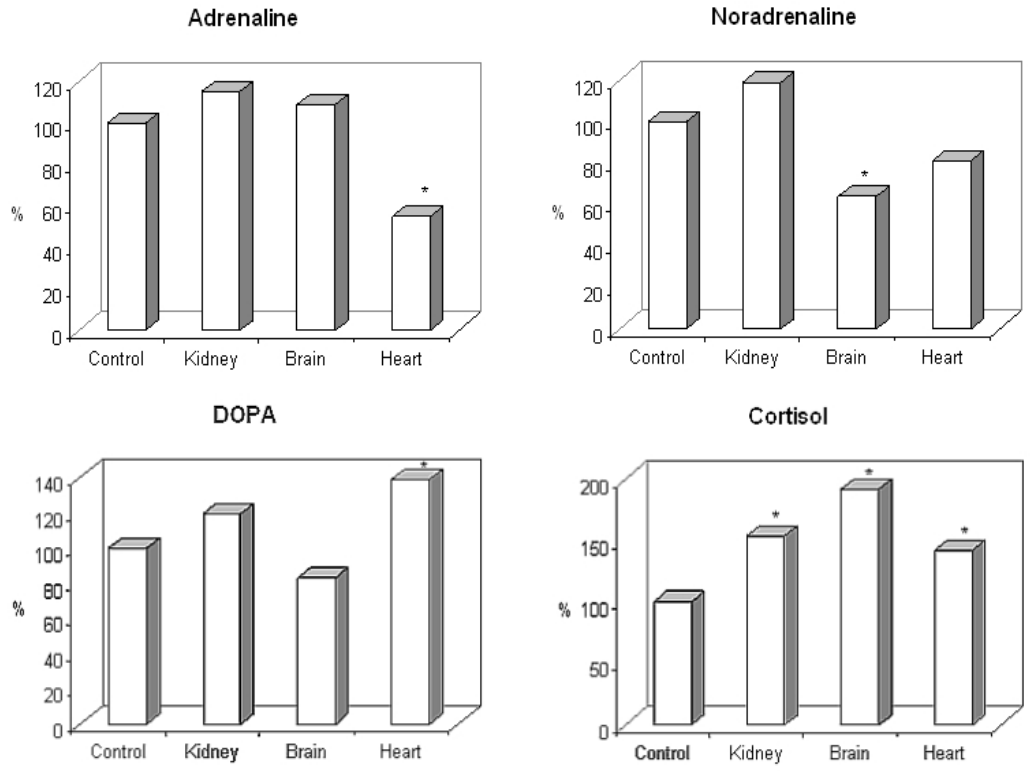
Cortisol increases reliably ^{59}FeTf binding to cell membranes of all organs tested, the effect is most pronounced in cell membranes isolated from brain brain (by 92%, $p < 0.001$), (Table 1 and Figure 1).

Table 1 ^{59}FeTf binding to cell membranes, isolated from rat kidney, brain and heart

Hormones (10^{-6} M)	Kidney g Fe x 10^{-9} (x ± SD)	Brain g Fe x 10^{-9} (x ± SD)	Heart g Fe x 10^{-9} (x ± SD)
Without agents	73.31 ± 12.79 (n=16)	84.88 ± 32.59 (n=53)	62.24 ± 5.56 (n=10)
Adrenaline	84.48 ± 20.15 (n=12)	92.86 ± 32.10 (n=20)	35.87 ± 11.12* (n=10)
Noradrenaline	86.98 ± 24.15 (n=12)	54.67 ± 16.23* (n=20)	50.64 ± 15.90 (n=10)
L-DOPA	86.96 ± 25.24 (n=12)	70.49 ± 20.88 (n=20)	86.50 ± 13.75* (n=10)
Cortisol	113.06 ± 30.60* (n=12)	162.57 ± 41.35* (n=20)	88.61 ± 10..06* (n=10)

* - p<0.001, n – number of samples, ** - Binding specifically ^{59}FeTf as g Fe x 10^{-9}

Figure 1. Different effect of hormones (%) on the ^{59}FeTf binding to cell membranes, isolated from rat kidney, brain and heart



Control – ^{59}FeTf binding without hormones (100 %).* - p<0.001

Discussion

Iron (Fe) sequestering molecules, such as ferritin, Tf, lactotransferrin, melanotransferrin, hemosiderin and heme can serve as cytoprotectants against metal-mediated oxidant damage. Rapid drop in serum iron concentration is associated with stress conditions (Fitzsimons and Levine, 1983). In states of infection (Van Snike et al. 1974), inflammation (Ward et al.2005) and in the treatment of Fe deficiency anemia with Fe supplementation (.Bring et al. 2008) Tf is saturated with metal, which can lead to overloading the cells with metal (Nappi and Vass, 2000). Tissue homeostasis of Fe is regulated by control of the process of connecting the FeTF to cell membrane receptors It is known that catecholamines interact with Fe and exert an neuroprotective effect (norepinephrine) (Paris et al. 2005) or cause degenerative changes (dopamine, an product of DOPA catabolism), (Stokes et al. 1999), but no studies whether these hormones alter the binding of FeTF to cell membrane receptors.

The cells can reduce Fe in diferric transferrin at the cell surface membrane receptors and this reduction reaction depends on the transmembrane electron transport system. Reduction of external diferric transferrin is accompanied by oxidation of internal NADH which indicates that the transmembrane enzyme is an NADH diferric transferrin reductase (Löw et al. 1986). The lowering of ^{59}FeTf binding in the presence of adrenaline (heart cell membranes) or noradrenaline (brain cell membranes), (Table 1 and Figure1), might be due to competition between ^{59}FeTf and hormones for electrons, transported with the membrane ferrireductase). Another possibility is the loss of Fe from transferrin (Freetstone et al. 2000) or the forming of a stable complex ^{59}FeTf - hormone (Ryan et al. 1993).

Heart cells have the ability to accumulate transferrin-bound Fe via the transferrin receptor and non-transferrin bound Fe probably via the L-type Ca2+ channel and the divalent metal transporter1 (Qian et al. 2007). DOPA and adrenaline are synthesized by one and the same metabolic chain, but exerted contrary effect on the ^{59} FeTf binding to the heart cell membrane receptors probably because of some differences in molecular structure (Table 1 and Figure 1).

DOPA increased ^{59}FeTf binding to the heart cell membranes probably as an iron chelator (Table 1 and Figure1), but the exact mechanism still remains unclear. Increased binding of ^{59}FeTf in the presence of L-DOPA (Table 1 and Figure1) could lead to an overload of Fe in certain circumstances and to participate important role in a number of heart disorders.. Adrenaline was shown to form colored complexes with both iron and copper at pH 7.0. Adrenaline inhibited ^{59}FeTf receptor interaction (Table 1 and Figure 1) and could protects cardiac cells from iron overload and oxidative changes. (Ryan et al. 1993).

Oxidation of the dopamine molecule produces a reactive quinone moiety that is capable of covalently modifying and damaging cellular macromolecules. Macromolecular damage, combined with increased oxidant stress, may trigger cellular responses that eventually lead to cell death. Reactive quinones have long been known to represent environmental toxicants and, within the context of dopamine metabolism, may also play a role in pathological processes associated with neurodegeneration. The quinone formation occurs spontaneously, can be accelerated by metal ions (manganese or iron) (Stokes et al. 1999). Norepinephrine reduce dopamine-dependent Fe toxicity in cells derived from the substantia nigra. Our results suggest that noradrenaline inhibits the binding of ^{59}FeTF to receptors on brain membranes (Table 1 and Figure 1), which coincides with the observation that the hormone can have neuroptotective effects by inhibition of the initiation of metal-free radical processes (Paris et al. 2005).

The classical genomic mechanisms of steroid hormone action cannot account for their rapid cellular effects. Membrane-bound steroid receptors have been partially characterized, but many rapid steroid effects occur in the absence of steroid-protein binding. By small-angle X-ray diffraction was found that cortisol increased overall bilayer with 3-4 A, a change that could modulate the structure and function of integral membrane proteins independent from steroid effects on the genome (Golden et al. 1998; Falkenstein et al. 2000)**.** Neurodegenerative disorders include a

number of different pathological conditions, which share similar critical metabolic processes, such as protein aggregation and oxidative stress, both of which are associated with the involvement of metal ions (Sayre et al. 1999; Hill et al. 1995)**.** Cortisol increased ^{59}FeTf binding to the cell membranes from: brain (with 92%, $p < 0.001$) which makes it possible involvement in brain degenerative processes involving Fe (Table 1 and Figure 1)..

The present results obtained in experiments *in vitro* proposed some additional data about the changes in iron homeostasis in conditions with elevated catheholamines and cortisol (Plunkett et al. 1997).

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