CHANGES IN CARDIAC NUCLEOTIDE METABOLISM IN HUNTINGTON’S DISEASE

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ABSTRACT
Huntington’s disease (HD) is a monogenic neurodegenerative disorder with a significant peripheral component to the disease pathology. This includes an HD-related cardiomyopathy, with an unknown pathological mechanism. In this study, we aimed to define changes in the metabolism of cardiac nucleotides using the well-established R6/2 mouse model. In particular, we focused on measuring the activity of enzymes that control ATP and other adenine nucleotides in the cardiac pool, including eNTPD, AMPD, e5’NT, ADA and PNP. We employed HPLC to assay the activities of these enzymes by measuring the concentrations of adenine nucleotide catabolites in the hearts of symptomatic R6/2 mice. We found a reduced activity of AMPD (12.9 ± 1.9 nmol/min/mg protein in control; 7.5 ± 0.5 nmol/min/mg protein in R6/2) and e5’NT (11.9 ± 1.7 nmol/min/mg protein in control; 6.7 ± 0.7 nmol/min/mg protein in R6/2). Moreover, we detected an increased activity of ADA (1.3 ± 0.2 nmol/min/mg protein in control; 5.2 ± 0.5 nmol/min/mg protein in R6/2), while no changes in eNTPD and PNP activities were detected. Analysis of cardiac adenine nucleotide catabolite levels revealed an increased inosine level (0.7 ± 0.01 nmol/mg dry tissue in control; 2.7 ±0.8 nmol/mg dry tissue in R6/2) and a reduced concentration of cardiac adenosine (0.9 ± 0.2 nmol/mg dry tissue in control; 0.2 ± 0.08 nmol/mg dry tissue in R6/2). This study highlights a decreased rate of degradation of cardiac nucleotides in HD mouse model hearts, and an increased capacity for adenosine deamination, that may alter adenosine signaling.

Keywords: Huntington’s disease, HD-related cardiomyopathy, adenine nucleotides catabolism, adenosine deaminase

INTRODUCTION
Huntington’s disease (HD) is an inherited neurodegenerative disorder that is caused by expanded CAG repeats within exon-1 of the huntingtin gene (HTT). These translate into a polyglutamine stretch (polyQ) in the HTT protein; for a recent review see (1). HTT is a 348-kDa multidomain protein that is normally expressed in various mammalian tissues, with the highest concentrations appearing in the brain and tests (2). Although HD manifests with a major pathology within the CNS (Central Nervous System), there is mounting evidence that HD is a
multi-system disorder (Mielcarek M, 2015). In fact, recent studies with HD mouse models confirmed an increased risk of heart contractile dysfunction and dilated cardiomyopathy (4). Typically, any type of cardiomyopathy may be associated with a reduction in activity of pathways producing adenosine-5'-triphosphate (ATP) and regenerating ATP (5).

It is well-known that nucleotides and nucleosides are key components of energy metabolism in the heart and other organs. Furthermore, they play an important role as cardiac extracellular signaling molecules, via the activation of purinergic receptors, classified as P1 for adenosine or P2 for ATP (6).

The cardiac pool of ATP and other adenine nucleotides is controlled by both extracellular and intracellular enzymes. Extracellular examples include ecto-nucleoside triphosphate diphosphohydrolase (eNTPD), an enzyme converting adenosine-5'-triphosphate (ATP) to adenosine-5'-diphosphate (ADP) and to adenosine-5'-monophosphate (AMP). Additionally, ecto-5'-nucleotidase (e5'NT), converts AMP to adenosine. There are also several intracellular enzymes: adenosine-5'-monophosphate (AMP) deaminase (AMPD) converts AMP to inosine-5'-monophosphate (IMP); adenosine deaminase (ADA) is responsible for the degradation of adenosine to inosine; purine nucleoside phosphorylase (PNP) is responsible for inosine to hypoxanthine degradation (7). Importantly, the adenine nucleotide catabolites that are produced by the reactions mentioned above may play a role in the regulation of cardiac function.

In this study, we defined a role for cardiac nucleotides in a metabolism imbalance in the symptomatic R6/2 mouse model. We examined the major intra- and extracellular enzyme activities involved in the degradation of cardiac adenine nucleotides by measuring the concentration of their cardiac catabolites, such as inosine and adenosine.

**MATERIALS AND METHODS**

The R6/2 mouse line is a commonly-used model of HD that is transgenic for a mutated N-terminal exon 1 HTT fragment. R6/2 mice were maintained and genotyped as previously described (9) and all experimental procedures performed on mice were conducted under a project license from the Home Office, UK, and approved by ethical committee at Imperial College London. In order to obtain heart samples, mice underwent terminal anaesthesia and heart tissues were freeze-clamped and stored in liquid nitrogen. Next, heart samples were homogenized in buffer containing: 150 mM KCl, 20 mM Tris, 1 mM EDTA, 1 mM dithiothreitol (pH 7.0) plus 0.1% (v/v) Triton X-100. The incubation was carried out as described previously (6). Protein concentrations were measured using a standard Bradford
The conversion of substrates into products was measured using HPLC, as described previously (10). The results are presented as amount of products in nmol/min/mg protein.

To measure adenine nucleotide catabolite levels, heart samples were placed for 24 hours in a freeze dryer at -55 °C. Next, freeze-dried tissue fragments were extracted with 0.4 M perchloric acid in a 1:10 ratio followed by neutralization with 2 M KOH. Nucleotide levels were measured by HPLC, as above. Results are shown as nmol/mg dry tissue.

The values are presented as mean ± SEM. Statistical analyses were performed using paired Student t tests. A p-value <0.05 was considered as significant.

RESULTS

The activities of the cardiac eNTPD, AMPD, e5’NT, ADA and PNP enzymes in an R6/2 HD mouse model are presented in Figure 1. We observed no statistically significant difference in eNTPD and PNP activity, between HD mouse model hearts and their control littermates. By contrast, AMPD activity was significantly decreased. An analysis of e5NT and ADA activities revealed their reduced activity in the HD mouse model, in comparison to the control mice. Finally, we found an approximately four times higher activity of cardiac ADA enzyme in these symptomatic mice.

Next, we measured cardiac adenine nucleotide catabolite levels (inosine, adenosine; Figure 2). We found a three times higher level of inosine in HD mouse model hearts. However, the level of cardiac adenosine was significantly reduced. Overall, we found an imbalance in the cardiac adenosine level in HD, that might be partially caused by a malfunction of the main cardiac enzymes that control its metabolism. The imbalance leads to mitochondrial dysfunction and consequently to HD-related cardiomyopathy.

DISCUSSION

In order to investigate the mechanism leading to HD-related cardiomyopathy, we validated the activities of a number of enzymes that are believed to be involved in the homeostasis of cardiac nucleotide metabolism. For this purpose, we analyzed heart samples from the well-described R6/2 mouse model that were fully symptomatic for neurological characteristics of HD. We found reduced AMPD and e5’NT enzymatic activities, increased ones for ADA, while eNTPD and PNP activities remained unchanged. It has been shown that
mutant HTT may interact with proteins involved in gene transcription, intracellular signaling and trafficking (11). Thus, we suggest that mutant HTT could disrupt cardiac nucleotide metabolism and purinergic signaling on a transcriptional level. This may result in significant alterations of the enzymes involved in adenine nucleotide metabolism.

Changes in purinergic signaling, or imbalances in adenine nucleotide catabolism enzymes, are crucial for maintaining cardiac energy homeostasis under pathological conditions. It is known that AMPD plays a role in the regulation of the adenine nucleotide pool as well as in the synthesis of guanine nucleotides (12). A reduced AMP deaminase activity in HD hearts could be indicative of a suppressed cardiac metabolism and a decreased demand for guanine nucleotides. Moreover, these hearts are characterized by a reduced activity of an enzyme involved in adenosine production (e5'NT) and an elevated activity of an enzyme responsible for adenosine degradation (ADA). These findings are in line with the observed reduction of cardiac adenosine - and elevated inosine - in HD hearts. One may conclude that a reduced e5'NT activity could lead to a shift in the extracellular versus cytosolic pool of adenosine, although the cytosolic fraction of adenosine has not been studied to date.

In the heart, adenosine acts as a signaling molecule and is involved in many processes like coronary vasodilatation, tachycardia and stimulation of the atrioventricular node conductance. Adenosine inhibits neutrophil activation, reduces infarct size and preserves endothelial function during inflammation, as well having potential roles in preconditioning via activation of A1 and A3 receptors (13). In fact, administration of adenosine metabolism inhibitors resulted in an improvement of cardiac function (14). Our study strongly indicates that changes in the enzymatic activities of molecules involved in nucleotide catabolism (i.e. that reduce adenosine formation and favor degradation) may result in an imbalance in cardiac adenosine concentration and signaling. Thus, these enzymes may play an important role in cardiac pathology in HD pre-clinical settings (Figure 3) and may be considered to be attractive therapeutic targets.

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FIGURE LEGENDS

**Figure 1.** Activities of enzymes involved in cardiac adenine nucleotide catabolism. A. eNTPD activity. B. AMPD activity C. e5’NT activity. D. ADA activity. PNP activity. Data presented as mean ± SEM; n=5*, p<0.05, ***p<0.001.

**Figure 2.** Cardiac concentration of catabolites of adenine nucleotides. A. Inosine concentration. B. Adenosine concentration. Data presented as mean ± SEM; n=5. *p<0.05.

**Figure 3.** A schematic overview of the pathogenic processes involved in nucleotide catabolism in HD pre-clinical settings.
REFERENCES

Figure 1
Figure 2
Figure 3

ATPase - adenosine 5'-triphosphatase
eNTPD - ecto-nucleoside triphosphate diphosphohydrolase
AMPD - adenosine-5'-monophosphate deaminase
e5NT - ecto-5'-nucleotidase
eADA - ecto-adenosine deaminase
ADA - adenosine deaminase
PNP - purine nucleoside phosphorylase