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Different expression of the catalytic alpha subunits of the AMP activated protein kinase - an immunohistochemical study in human tissue

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Summary. AMPK is an ubiquitously distributed multienzyme complex. It is an important energy sensor and regulator of cellular metabolic activity. In this study we analyzed for the first time the cellular distribution of the catalytically active subunits AMPKα1 and α2 in different human tissues by immunohistochemistry. We found different expression patterns for both isoforms. AMPKα2 expression clearly dominates in skeletal myocytes and cardiomyocytes, whereas AMPKα1 dominates in a number of secreting cells, like mammary glands, islets of langerhans and cells of the colon crypts.

Key words: AMPK, Immunohistochemistry, Secretion

Introduction

The AMP-activated protein kinase (AMPK) is a central protein in the regulation of energy metabolism of the whole body (Lage et al., 2008; Dzamko and Steinberg, 2009). AMPK is a heterotrimer consisting of three subunits with different isoforms (α1, α2, ß1, ß2, γ1, γ2, γ3). The α subunits confer kinase activity, whereas the ß and γ subunits have structural and regulatory functions (Carling, 2004; McGee and Hargreaves, 2010). Different AMPK holoenzymes exist which differ in their subunit composition. In skeletal muscle the main AMPK-isoform contains the subunits α2ß2, as was shown by immunoprecipitation experiments (Wojtaszewski et al., 2005). An earlier investigation by northern and western blot showed an ubiquitous but weak distribution of AMPKα1 in all tissues, whereas high levels of AMPKα2 were only reported for heart muscle, liver and skeletal muscle. In other tissues like brain, lung or kidney AMPKα2 was weakly expressed (Stapleton et al., 1996). The exact distribution of AMPK subunits in special cell types of different tissues is still unknown. Moreover, it is unknown if the two α isoforms comprise different functions or if they are able to compensate for each others acitivity. By scanning different human tissues with antibodies directed against the α1 and ß2 subunits of AMPK we were able to allocate the expression patterns of AMPKα2 and α1 in different human tissues to distinct cell types. The AMPKα1 and α2 distribution patterns provide new functional aspects for both subunits.

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Material and methods

Immunohistochemistry

Small tissue stripes were collected from macroscopically healthy regions of surgically removed tissues. The experiments were performed in accordance with the German ethical requirements and were approved by the ethical comission of the University of Göttingen (November, 2009). For immunohistochemistry staining formalin fixed and paraffin embedded tissue slices were used. For antigen retrieval the slices were incubated in a steamer in target retrieval buffer pH 6.1 (DAKO, Hamburg, Germany) for 40 min, washed with TBS buffer (0.5% TWEEN 20), incubated with DAKO peroxidase blocking reagent (DAKO) for 12 min, blocked with DAKO Real Antibody Diluent (DAKO) and incubated with monoclonal rabbit anti

AMPKα1 (0.43 μg/ml) (Clone Y365, Epitomics, Burlingame, CA) or goat polyclonal AMPKα2 (2 μg/ml) (A-20, Santa Cruz Biotechnology, Santa Cruz, CA), overnight at 4°C. For signal amplifying the DAKO EnVision Dual Link system-HRP (DAKO) (RT, 30 min) were used for AMPKα1 and for AMPKα2 Goat Probe (15min,RT) (Promark Goat Micro-polymer detection Kit, BIOCARE Medical, Concord, CA) followed by Goat Polymer-HRP (15 min, RT) (Promark Goat Micro-polymer detection Kit, BIOCARE Medical) were used. The signal was developed using DAKO Liquid DAB-Substrate (DAKO). Nuclei were visualized by Hämalaun staining. For all investigated tissues negative controls were made. In the negative controls the primary antibody was substituted by Rabbit Immunoglobin Fraction (Solid-Phase absorbed) (DAKO) or Goat Serum (DAKO) in the same protein concentration as the primary antibody. With this method no staining could be found in any negative controls.

Western blotting

40 μg of protein extract were subjected to SDS-Polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The membrane was blocked in TBS buffer containing 5% BSA. For the detection of the AMPKα1 and α2 proteins the same antibodies were used as for immunohistochemistry (each in a dilution of 1:1000). HRP-conjugated secondary antibodies were used. Luminescence was detected using Super Signal West Femto Chemiluminescent reagent (Pierce, Rockford, Il, USA) on an LAS-4000 Luminescent Image Analyzer (FUJIFILM, Europe).

Results

The immunostaining results for AMPKα1 and α2 are summarized in Table 1 for the analyzed human tissues. The most striking differences in the expression pattern of the two subunits can be found for cardiomyocytes and myocytes from striated muscles. Both muscle cell types show a strong cytoplasmatic staining for AMPKα2 and no staining for AMPKα1 (Fig. 1A,B). This striking difference for AMPKα2 and α1 exprression was also confirmed by western blotting (Fig. 2). Here, a very weak AMPKα1 band was detected in comparison with a strong α2 band in lysates from heart tissue.

On the contrary, a reversed distribution of subunits was found in mammary glands (Fig. 1H) and the cells in the crypts of the gut (Fig. 1G). Here we found a strong staining for α1 in the lining cells of the crypts and the cells of the mammary glands, whereas AMPKα2 could not be detected at all.

A different staining pattern for α1 and α2 could also be found in the pancreas (Fig. 1C). Cells of islets of langerhans showed a stronger staining of AMPKα1 than the surrounding acini cells. In contrast, the AMPKα2 staining is stronger in the acini cells than in the islets of Langerhans.

Less pronounced differences in the staining patterns between AMPKα1 and α2 were found in lymphocytes of tonsils (Fig. 1J) and the thyroid gland (Fig. 1L). In tonsils the lymphocytes showed a weak α1 staining and no α2 staining, whereas in the epithelial cells of the thyroid follicels only a very weak α1 staining could be detected.

No differences in the staining pattern of AMPKα1 and α2 could be found in the kidney (Fig. 1E), the stomach (Fig. 1D), the lung (Fig. 1I), the liver (Fig. 1F) and the parotid gland (Fig. 1K). A strong staining for both subunits was observed in the cells of the gastric glands and epithelial cells of the kidney tubuli. In the lung, the lining cells of the bronchioli showed a moderate staining for AMPKα1 and AMPKα2. In the liver, hepatocytes were weakly stained for AMPKα1 and α2. A strong to moderate staining for both subunits could be found in the ducts of the parotid gland.

Most of our results are in agreement with formerly published findings in different animal tissues concerning distribution of AMPKα1 and α2 subunits done by northern and western blot (Stapleton et al., 1996) but there are also some discrepancies. Stapleton et al. (1996) found a weak but equal expression of α1 in heart, brain, lung, liver skeletal muscle and kidney, for α2, in contrast, they found a very high expression in heart, liver and skeletal muscle and only weak or no expression in the other tissues.

In some published cell culture experiments the α2 subunit was also found in the nucleus (Salt et al., 1998; Bronner et al., 2004; Kodiha et al., 2007). With our staining method a positive staining was predominantly found in the cytoplasm for α1 and α2 (Fig. 3).

Table 1. Staining patterns of AMPKα1 and α2.

Tissue	Cell types	Staining pattern		
		Strong	middle-weak	No
Heart	Cardiomyocytes	α2		α1
Striated Muscle	Myocytes near thyroid gland	α2		α1
Pancreas	Langerhans islet cells	α1	α2	
	Pancreatic acini cells	α2	α1	
Stomach	Cells of the gastric glands	α1 α2		
Kidney	Epithelial cells of the tubuli	α1 α2		
Liver	Hepatocytes		α1 α2	
Gut	Cells of the crypts	α1		α2
Breast	Cells of the mammary glands	α1		α2
Lung	Lining cells of the bronchioli	α1	α2	
Tonsile	Immune cells		α1	α2
Parotid gland	Epithelial cells of the ducts		α1 α2	
Thyroid gland	Follicular epthelial cells		α1	α2

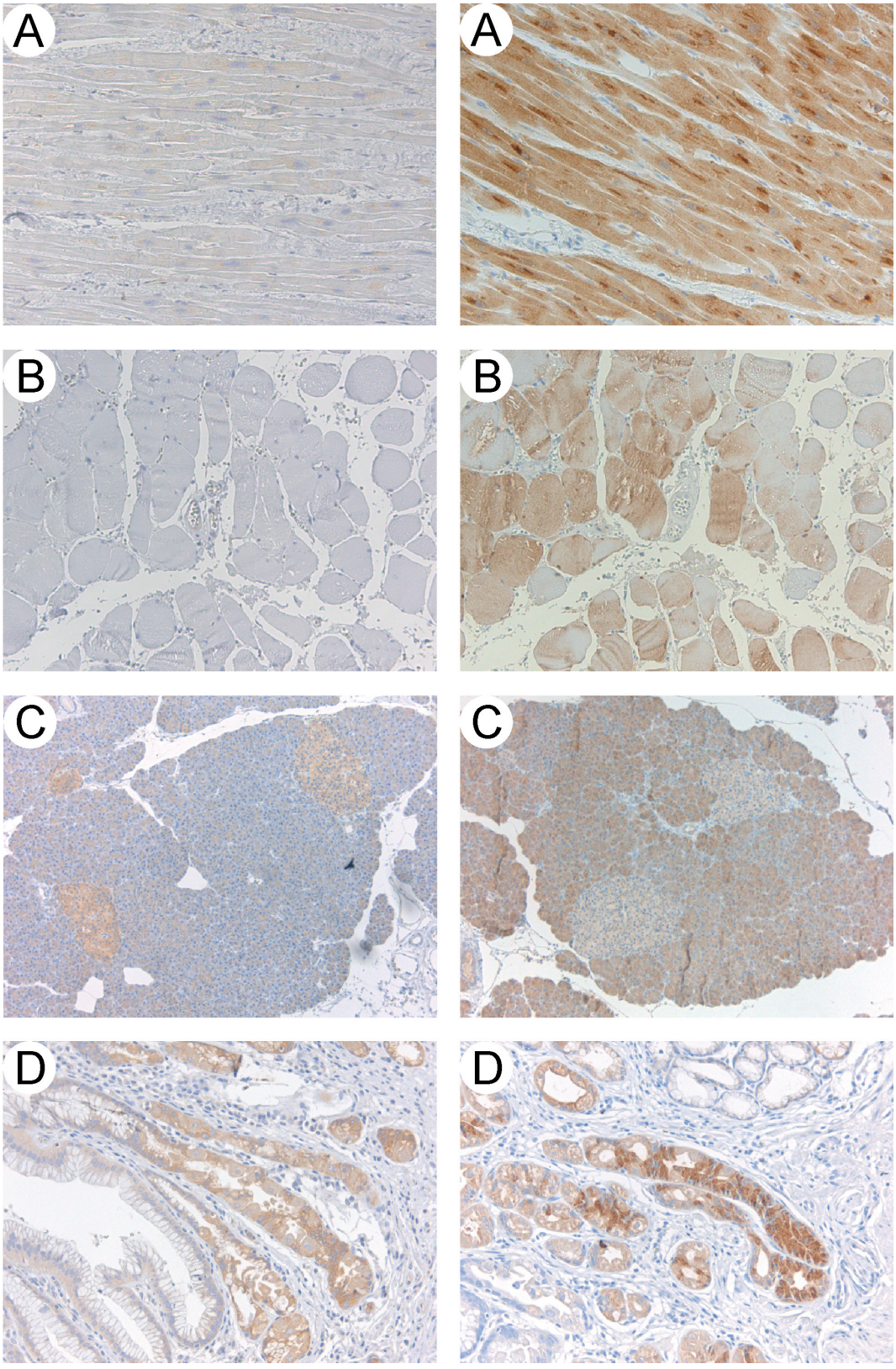

Fig. 1. a. Comparison of AMPKα1 and α2 in different human tissues by immunohistochemistry. Staining for α1 subunit is shown on the left and for α2 on the right panel. A: Heart, B: Striated muscle, C: Pancreas, D: Gastric glands. x 200

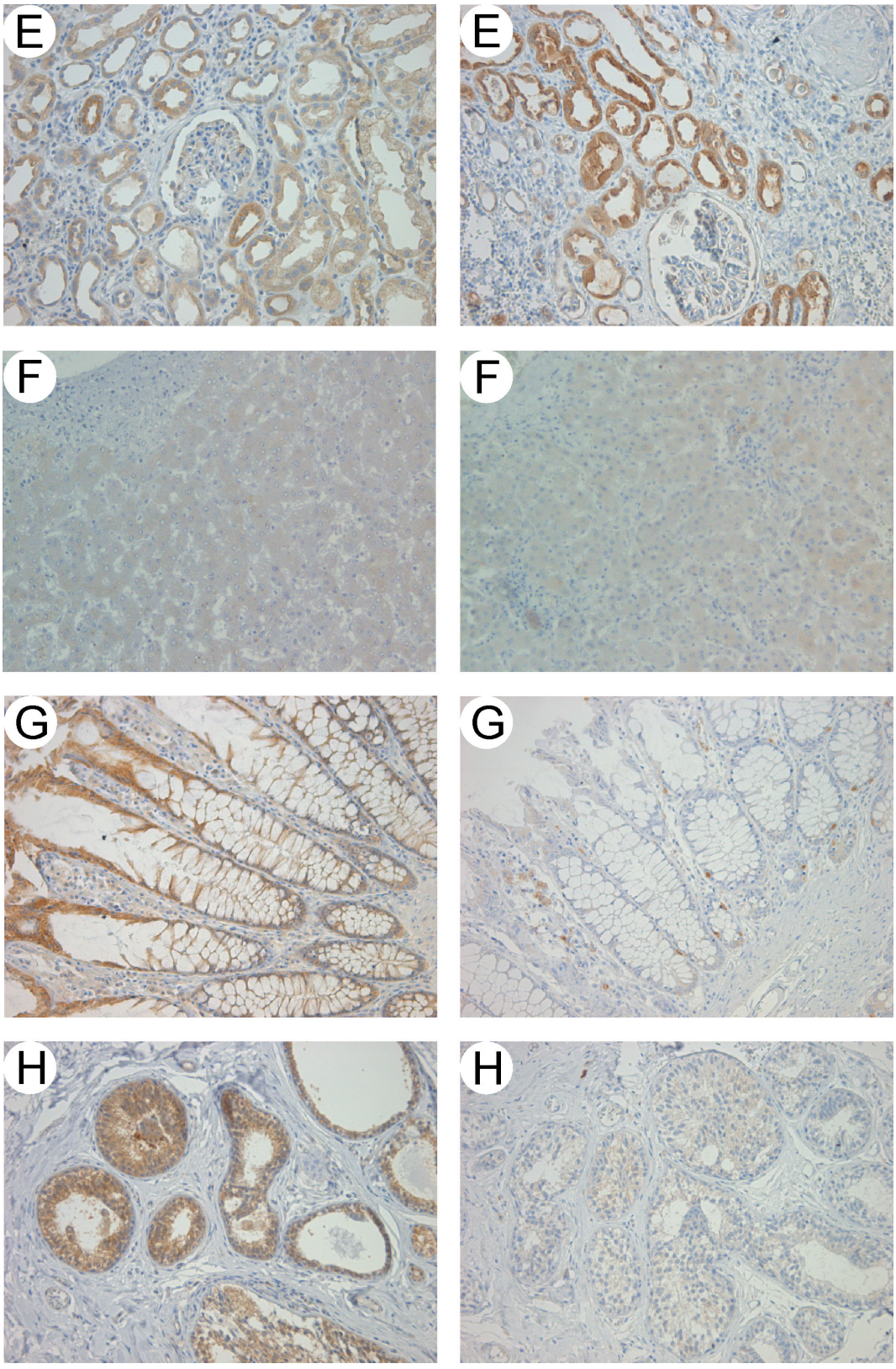

Fig. 1. b. Comparison of AMPKα1 and α2 in different human tissues by immunohistochemistry. Staining for α1 subunit is shown on the left and for α2 on the right panel. E: Kidney tubuli, F: Liver, G: Colon crypts, H: Mammary glands. x 200

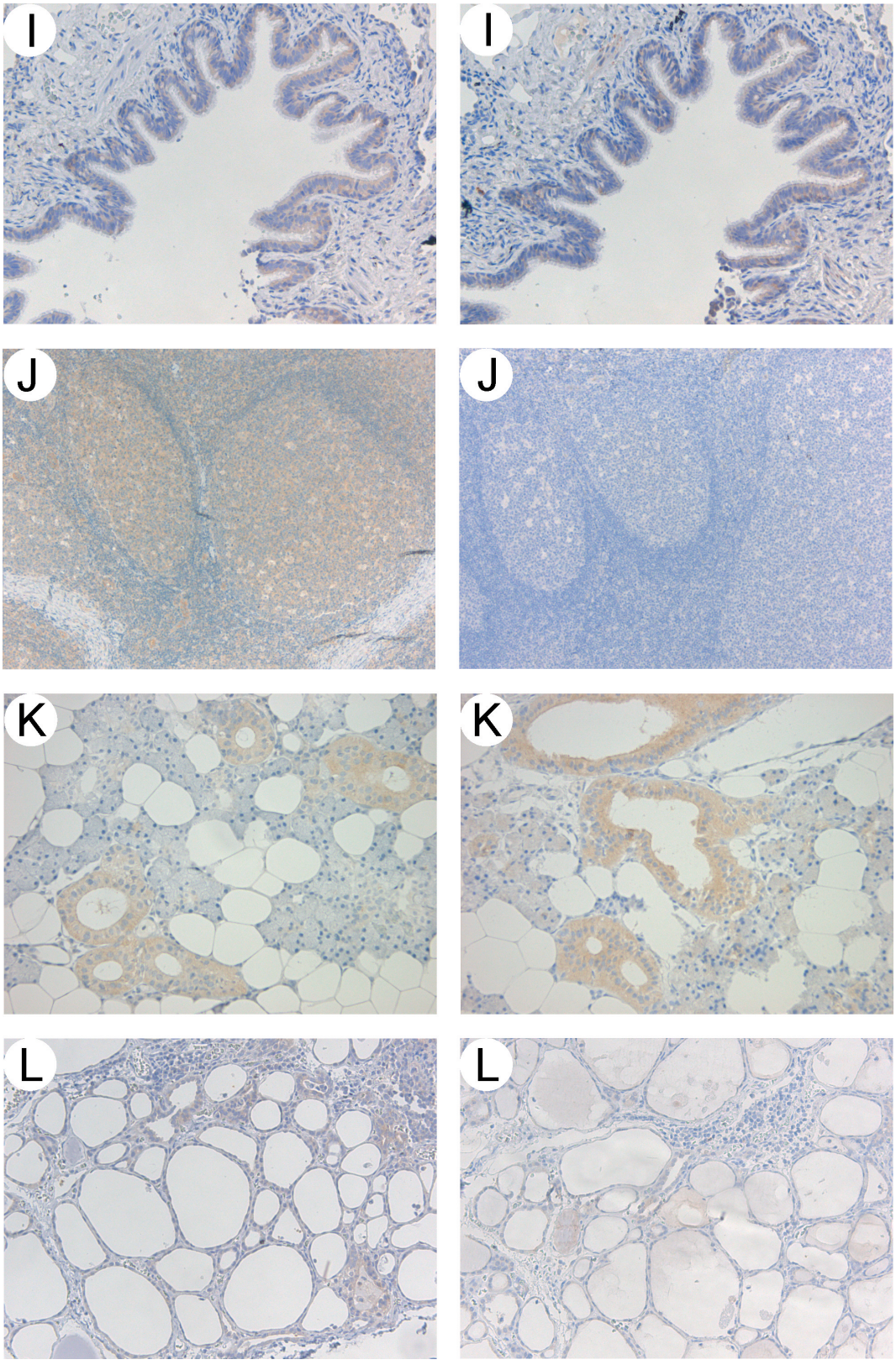

Fig. 1. c. Comparison of AMPKα1 and α2 in different human tissues by immunohistochemistry. Staining for α1 subunit is shown on the left and for α2 on the right panel. I: Bronchioli of the lung, J: Tonsile, K: Parotid gland, L: Thyroid gland. x 200

Discussion

In the present study we present an investigation of AMPKα1 and α2 distribution in different human tissues by immunohistochemistry. To the best of our knowledge, this is the first study of this kind performed in human tissue.

A formerly published study of α1 and α2 distribution in a panel of different tissues was done on rat tissue by northern and western blot (Stapleton et al., 1996). This study reported a weak but almost equal expression of α1 mRNA for each tissue examined (heart, brain, lung, liver, skeletal muscle, kidney, testis). Evaluation of α1 protein expression by western blot showed an α1 band for all analyzed tissues with a slightly more pronounced band for the heart. These results are difficult to compare with our immunostains, because in lung and kidney we found AMPKα1 staining for distinct tissue structures only. In contrast, the previously reported investigation of AMPKα1 expression by northern and western blotting with complete organ lysates provides an average amount through all cell types and therefore the results are not comparable with those from compact organs like heart or skeletal muscle, which mainly consist of one cell type. For compacter organs like heart, skeletal muscle and liver, α1 expression by northern and western blot has been reported to be almost equal. Our results for human tissue differ from this observation. This could be due to a higher sensitivity of the α1 western blot in comparison to the immunostaining, as we have also observed in our experiments (Fig. 2)

For AMPKα2, Stapleton et al. (1996) clearly showed dominating bands in heart, skeletal muscle and liver by northern and western blotting. Our strong immunohistochemical stains of cardiomyocytes and myocytes from striated muscle fit well with the predominance of α2 mRNA and protein expression as reported by Stapleton et al. However, in contrast to Stapleton et al. (1996), our immunostaining results for liver would predict a weaker mRNA and protein expression in comparison with heart and skeletal muscle. We cannot explain why our immunostaining in liver does not display the more equal α2 distribution between heart, skeletal muscle and liver reported by mRNA and protein expression analysis in the study by Stapleton et al. (1996). Different expression patterns between rat and human tissue may be a reason. We also speculate that the turnover rate of AMPKα2 is higher in liver cells than in heart or muscle cells.

The consistent results of a predominant α2 expression in cardiomyocytes and striated muscle myocytes in our study and in that of Stapleton et al. (1996) fits well with other published investigations which compare the function of α1 and α2 specifically in muscle lysates. In these publications a higher expression level of AMPKα2 and a specific increase in α2 activity through exercise has been shown in lysates from skeletal muscles (Wojtaszewski et al., 2000, 2005; Birk and Wojtaszewski, 2006) and also in heart tissue (Tian et al., 2001) by western blot and determination of the specific activities of the α1 and α2 subunits.

Another study by Conde et al. (2006) is concerned with the different AMPKα subunits in muscle. It examined the expression of LKB1 and phosphorylated Acyl-CoA Carboxylase (p-ACC) in various human tissues by immunohistochemistry. Both enzymes are directly linked to AMPK. LKB1 is an upstream kinase that phosphorylates both AMPKα subunits in vitro (Jansen et al., 2009). ACC is an important target protein that is activated by AMPK mediated phosphorylation and thereby facilitates fatty acid uptake and oxidation in the mitochondria.

Both cardiomyocytes and skeletal or striated myocytes exhibit a dominant expression of the α2 subunit as well as LKB1 and p-ACC expression, in the tissues studied by Conde et al. (2007) as well as in our study. This combination cannot be found in any other tissue studied in our experiments. Thus, this finding supports the thesis that the LKB1 regulated AMPKα2 isoform is predominantly involved in metabolic adaptation of muscle cells. A study with transgenic mice of deficient LKB1 activity showed that anoxia induced activation and phosphorylation of AMPKa2 and ACC were completely abolished, whereas AMPKα1 activity and phosphorylation were only moderately inhibited (Sakamoto et al., 2006).

The staining patterns we found for AMPKα1 distribution were surprising: While it was assumed that α1 is a more or less ubiquitously expressed subunit of AMPK (Stapleton et al., 1996), we found that especially cells with secretory functions had a remarkably high expression of AMPKα1. In crypts of the gut and mammary glands only α1, but no α2, was detectable. However, islets of Langerhans in the pancreas exhibited a much stronger signal for α1 than for the α2 subunit.

Our findings support the notion that AMPKα1 plays an important role in some secretory processes. This connection has previously been suggested by other groups. It has been shown that AMPK is involved in the regulation of progesterone secretion in rat granulosa cells (Tosca et al., 2005) which also have a very strong signal for AMPKα1. Sidani et al. (2009) reported a high

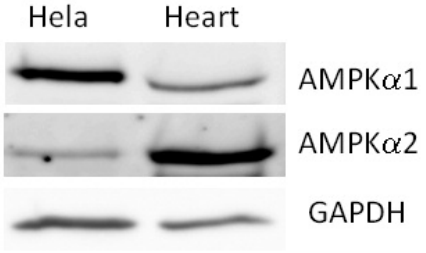

Fig. 2. Determination of AMPKα1 and α2 expression in human heart tissue in comparison to Hela-cells by western blotting. GAPDH serves as loading control. 40 μg protein lysate was loaded per lane.

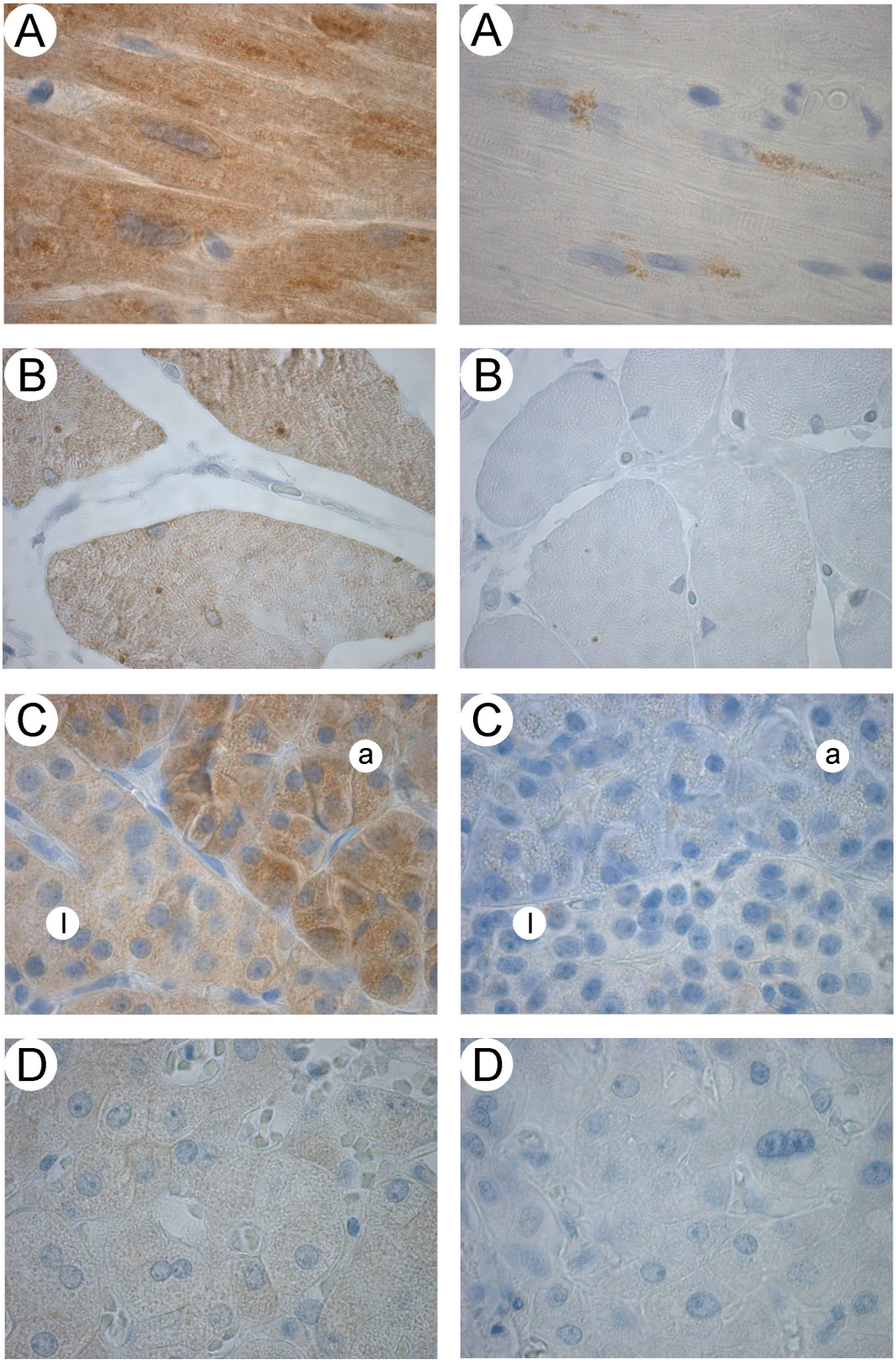

Fig. 3. Subcellular localization of the AMPKα2 subunit. Stainings for α2 are shown on the left and the appropriate negative controls on the right panel. Negative controls were performed using non immune serum instead of the primary antibody as described in the material and methods section. A: Heart with a non specific lipofuscin staining in the negative control, B: Striated muscle, C: Pancreas (I: islets of Langerhans, a: acini cells), D: Liver. x 1.000

expression of AMPKα1 in the parietal cells of rat stomach and that the ATP consuming transport of protons by these cells is dependent on the AMPK activity. An influence of AMPK on the vesicle movement in insulin secreting, cells has also been demonstrated (Tsuboi et al., 2003).

Our study provides new insight into AMPK distribution in human tissues and suggests an involvement of the AMPKα1 subunit in secretory functions of different glandular cells. Moreover, our investigation highlights different functions derived from the distribution patterns of the catalytically active subunits α1 and α2.

Conclusion

In this study we compared the AMPKα1 and α2 expression level in different human tissues by immunohistochemistry. A striking difference in the staining pattern of both subunits between muscle cells and a variety of secretory cells has been found. This supports the hypothesis that AMPKα2 is necessary for the energy metabolism of muscle cells, and raises the question whether AMPKα1 is involved in a common mechanism that regulates secretory processes.

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