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Poster presentation

Analysis of phosphodiesterase expression using quantitative real time PCR

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Basal intracellular guanosine 3',5'-cyclic monosphosphate (cGMP) levels and the pattern of cGMP accumulation and degradation after guanylyl cyclase stimulation are strongly influenced by the presence of different cGMP hydrolysing phosphodiesterases (PDEs).

At least 21 PDE genes have been identified and subgrouped into 11 families. PDE4, PDE7 and PDE8 preferentially hydrolyse adenosine 3',5'-cyclic monosphosphate (cAMP), while PDE5, PDE6 and PDE9 hydrolyse cGMP and PDE1, PDE2, PDE3, PDE10 and PDE11 can hydrolyse both cAMP and cGMP. These enzymes show a distinct regional, cellular and subcellular distribution pattern that can change during development and cellular activity.

We have determined the relative mRNA expression levels of the cGMP metabolising phosphodiesterases PDE5, PDE9 (cGMP specific), PDE2, PDE10 (hydrolyse both cGMP and cAMP) in 24 different human tissues using quantitative real time PCR. Pooled RNA samples derived from at least 4 donors were used for cDNA synthesis. Three housekeeping genes were used as internal standards. Primers were designed to amplify cDNA regions that are present in all known splicing isoforms, optimised and used for quantitative real time PCR (ABI PRISM 7900HT, SYBR Green detection method).

PDE10 mRNA showed the most striking differential distribution, with very high levels in caudate nucleus and n. accumbens, at least 20 fold lower levels in thalamus, cerebellum, hippocampus, various cortical regions and substantia nigra and generally very low expression in different peripheral tissues. PDE2 was highly expressed in spleen, caudate nucleus, cortex, hippocampus and nucleus accumbens with lower levels in adrenal gland, bladder, thalamus and hypothalamus. Of the 24 tissues examined, PDE5 showed highest expression levels in lung, followed by bladder, heart, small intestine, dorsal root ganglia and lower but significant levels in most other tissues. The levels of PDE9 mRNA showed less variation between different tissues. Most tissues showed between 30 and 70 % of the highest signal, which was found in bladder. Lowest levels of PDE9 mRNA were present in heart, liver and skeletal muscle (5–10% of the maximum signal).

Our data confirm and extend earlier studies using in situ hybridisation, Northern Blot or PCR analysis in rat, mouse and human tissues and are consistent with the distribution pattern for PDE10 protein in rat brain that was determined by immunohistochemistry [1].

References

1. Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, Lanfear J, Ryan AM, Schmidt CJ, Strick CA, Varghese AH, Williams RD, Wylie PG, Menniti FS: **Immuno-histochemical localization of PDE10A in the rat brain.** *Brain Res* 2003, **985:**113-126.