A direct method to visualise the aryl acylamidase activity on cholinesterases in polyacrylamide gels

ABSTRACT

A novel method to visualise the aryl acylamidase activity on cholinesterases in polyacrylamide gels has been developed.

INTRODUCTION

Background Cholinesterases (ChEs) are evolutionarily conserved type B carboxylesterase enzymes that share extensive sequence homology. In vertebrates two types of ChEs were identified based on their distinct substrate specificity and inhibitor sensitivity. The acetylcholinesterase (AChE; EC 3.1.1.7) specifically catalyses the hydrolysis of acetylcholine and is subjected to marked inhibition by its own natural substrate. In contrast, butyrylcholinesterase (BChE; EC 3.1.1.8) is capable of degrading a wider range of choline esters and is not inhibited by its substrate. AChE is selectively inhibited by BW 284c51, while BChE is specifically inhibited by tetraisopropylpyrophosphoramide (iso-OMPA). AChE is widely distributed in the nervous system and its role in rapidly terminating nerve impulse by hydrolysing acetylcholine in cholinergic synapses is well documented. BChE is produced in the liver and enriched in the circulation. In addition, it is also present in adipose tissue, intestine, smooth muscle cells, white matter of the brain and many other tissues. The exact physiological function of BChE is still elusive. It is generally viewed as a back up for the homologous AChE and to act as a scavenger for anticholinesterase compounds. The presence of ChEs in tissues that are not cholinergically innervated provides the most compelling evidence for the view that AChE and BChE may have functions, other than the termination of cholinergic neurotransmission. There is considerable body of evidence to suggest that ChEs may be involved in embryonic neural development, including a role in cell proliferation, differentiation and cell adhesion. ChEs may also have a causative/permissive role in various pathological conditions as exemplified by the overexpression of ChE genes in various types of tumours and presence of abnormal levels of ChEs with altered properties in Alzheimer's disease. The histochemical staining of esterase activity on ChE developed by Koelle and Friedenwald and modified by Karnovsky and Roots has been extensively used to elucidate the functions of ChEs, examine their tissue specificity, developmental alterations and pathological changes from many species. Other than the predominant choline esterase activity, ChEs also display a genuine aryl acylamidase (AAA) activity capable of hydrolysing the synthetic substrate o-nitroacetanilide into o-nitroaniline and acetate. Apart from being strongly inhibited by choline esters and classical ChE inhibitors, this AAA activity is susceptible to selective inhibition by 5-hydroxytryptamine (serotonin). The characteristic feature of the AAA activity associated with human serum BChE is its several fold activation by tyramine. The natural substrate or the precise physiological role of the AAA activity on ChEs is not known. Studies on the tissue specific and developmental regulations/alterations of AAA on ChEs have not been attempted so far partly due to the lower specific activity of the AAA activity on ChEs and mainly due to the absence of a specific method to visualise this activity. In the present paper, a method to visualise the AAA activity on ChEs in polyacrylamide gels using human serum BChE and electric eel AChE as models is described.

CONCLUSION

This study describes a novel method to visualise the AAA activity on ChEs in polyacrylamide gels. The method has been shown to be sensitive and also can selectively detect either of the ChE's AAA activity. Use of this method to visualise AAA activity in tissue sections, however, needs further refinement/modifications to enhance the sensitivity. This is because, any tissue, at a particular locus might not have AAA activity to the extent of 0.07 U. Nevertheless, this study is the first successful attempt to visualise the AAA activity on cholinesterase in vitro.