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Activation of myelin-associated cholesteryl ester hydrolase in developing rat brain

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Short-term regulation of rat brain cholesteryl ester hydrolase (CEH) by protein kinases is described. CEH was activated 280–340% in the presence of Mg^{2+}-ATP and this inhibition was partially abolished by rabbit skeletal muscle protein kinase inhibitor or chlorpromazine, a phospholipid interacting drug, suggesting the involvement of cAMP-dependent protein kinase and protein kinase C, respectively. However, the involvement of other kinases cannot be ruled out. In developing rat brain, CEH activity per unit brain weight closely correlated with myelination. During the premyelination period (5 days postnatal), significantly higher activation ($P < 0.001$) of CEH was observed by cAMP-dependent protein kinase or protein kinase C, when compared to activation observed during the period of active myelination (20 days postnatal). These results indicate that CEH in rat brain is tightly regulated and closely related to myelination.

Unesterified cholesterol is a major constituent of the myelin membrane in adult mammalian brain[15]. Several investigators have reported transient increases in cholesteryl esters (CE) in the immature CNS prior to the onset of myelination, followed by a decrease as myelination proceeded[6]. Substantial increases in CE levels have also been reported in demyelinating diseases such as multiple sclerosis and Wallerian degeneration. These observations suggest an important role for CE metabolism in myelination and demyelination.

Eto and Suzuki have demonstrated the presence in normal rat brain of a cholesterol esterifying activity[5] and a cholesteryl ester hydrolase (CEH), which catalyzes hydrolysis of cholesteryl esters[5]. In rat brain, at least 3 distinct CEH enzymes have been identified, differing in subcellular localization, pH optima and response to detergents[8]. One of these, a CEH with optimal activity at pH 6.6, is not only associated with myelin but appears to correlate with myelination[7]. Activity of this CEH is decreased in Quaking mice and Jimpy mice, which have primary defects in myelin synthesis[9]. CEH activity is also decreased in brains of multiple sclerosis (MS) patients[17], in cerebrospinal fluid of patients with acute MS[16] and during Wallerian degeneration[13]. Thus, accumulation of CE during demyelination may be due to decreased CEH activity.

Whereas regulation of CEH by reversible phosphorylation has been reported in adrenal cortex[2], corpus luteum[4], testis[1] and liver[11], mechanisms for regulation of brain CEH have not been reported. The present study provides evidence for activation of rat brain CEH by cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) and suggests correlation of that activation with myelination.

Rats weighing 200–300 g were sacrificed by decapitation. Brains were dissected, washed with 0.32 M sucrose and homogenized in 3–6 vols. of 0.32 M sucrose in a glass tube with a loose-fitting Teflon pestle. An aliquot of homogenate was solubilized in 0.1 N NaOH and protein was determined by the BCA procedure[18]. Hydrolysis of cholesteryl [1-^{14}C]oleate (NEN, Boston, MA) was determined in aliquots of brain homogenate (300–500 μg protein) by a modification of a procedure used for measurement of CEH in liver[11]. 100 mM potassium phosphate, pH 6.6 was used as the assay buffer, in order to inhibit endogenous phosphatase activity. Incubation time was 1 h. Reaction rate was linear with respect to time for 2 h, and with respect to protein, up to 1 mg/assay. Optimum pH for the homogenate activity was 6.6.

Activation of CEH by protein kinases A and C was determined as we have described previously for liver CEH[11]. CEH was assayed in the presence and absence of 1 mM $MgCl_2$, 5 mM ATP and 100 μM cAMP, cofactors for PKA, and in the presence and absence of 1 mM $CaCl_2$, 5 mM ATP, 20 μg/ml phosphatidyl serine and 4

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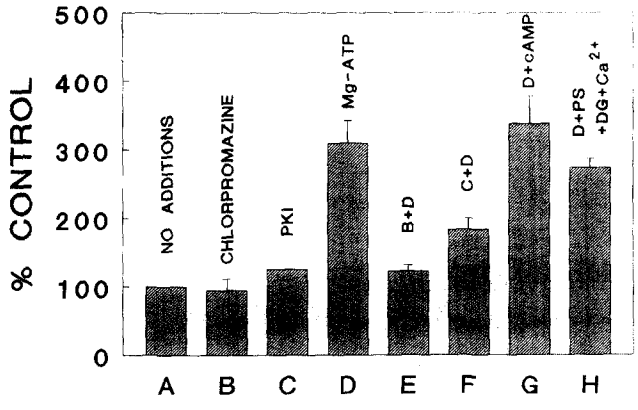

Fig. 1. Activation of CEH by cofactors for protein kinases. CEH was assayed as described in Materials and Methods in the presence of the following: A, no additions; B, 0.1 mM chlorpromazine (PKC inhibitor); C, 100 μg protein kinase inhibitor; D, 1 mM $MgCl_2$ and 5 mM ATP; E, 1 mM $MgCl_2$, 5 mM ATP and 0.1 mM chlorpromazine; F, 1 mM $MgCl_2$, 5 mM ATP and 100 μg protein kinase inhibitor; G, 1 mM $MgCl_2$, 5 mM ATP and 100 μM cAMP; H, 1 mM $MgCl_2$, 5 mM ATP, 5 mM $CaCl_2$, 20 μg/ml; phosphatidylserine, and 4 μg/ml diolein. Values shown are mean ± S.E.M. of 3 independent experiments and each experiment was done in triplicate. The basal activity varied from 0.74 to 1.11 nmol·h^{-1}·mg protein^{-1}.

μg/ml diolein, cofactors for PKC. The phosphatidyl serine and diolein emulsion was prepared as described by Beg et al.[3]. Rabbit skeletal muscle protein kinase inhibitor (200 μg/ml) or chlorpromazine (0.1 mM) were added to some assays to selectively inhibit PKA[21] or PKC[14], respectively.

CEH activity in brain homogenate was increased by 280–340% when assayed in the presence of Mg^{2+}-ATP, suggesting activation by protein kinase(s) (Fig. 1). Addition of skeletal muscle protein kinase inhibitor decreased the activation by 40% ($P < 0.005$), providing evidence for mediation by PKA. Presence of PKA has been demonstrated in rat brain homogenates[12]. However, since addition of cAMP produced no increase in activity over that seen with Mg^{2+}-ATP, dependence of activation on cAMP could not be demonstrated in the homogenate, probably due to the presence of endogenous cAMP. When 0.1 mM chlorpromazine, a phospholipid-interacting drug which inhibits PKC but not PKA, was included in the assay mixture, the activation decreased by 61% ($P < 0.05$) indicating a role for PKC in the activation process. When all cofactors required for PKC (Mg^{2+}-ATP, phospatidyl serine and diolein) were included in the reaction mixture, the activation observed was not significantly different from that observed with Mg^{2+}-ATP alone ($P > 0.05$). These results provide evidence for the short-term regulation of brain CEH by protein kinases. The role of PKA and PKC is clearly established, however, the involvement of other kinases in this activation process cannot be ruled out. Such short-term regulation of enzymes involved in cholesterol metabolism by multiple kinases has been observed for rat hepatic CEH[11] and also hepatic HMGCoA reductase[20], the rate-limiting enzyme in cholesterol biosynthesis.

To determine changes in CEH activity as a function of age and myelination, CEH was measured in brain from rats of various ages, corresponding to various stages of CNS myelination, i.e. premyelination (5- and 10-day-old), myelination (15–30-day-old) and postmyelination (40- and 60-day-old). As seen in Fig. 2, activity remained unchanged during the premyelination period, whereas

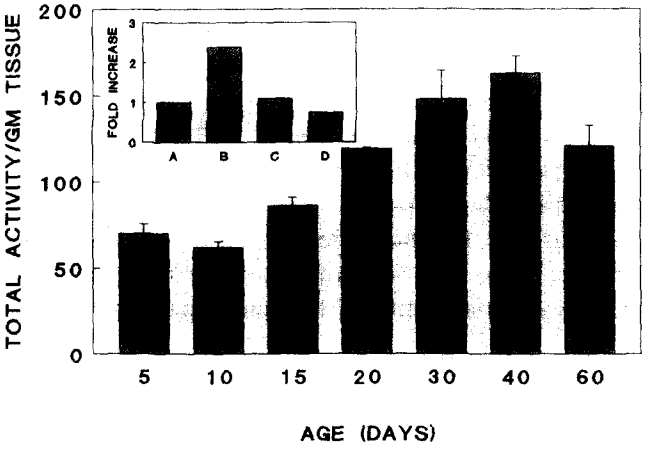

Fig. 2. Changes in CEH activity in developing rat brain. CEH activity was measured in brain homogenates as described in Materials and Methods. 300–500 μg protein was used in the reaction mixture. Four animals were used for each age group and values shown are mean ± S.E.M. The inset shows the fold increase in CEH activity during different developmental stages; A, premyelination period (5–10 days); B, myelination period (10–30 days); C, postmyelination period (30–40 days); D, adult (40–60 days).

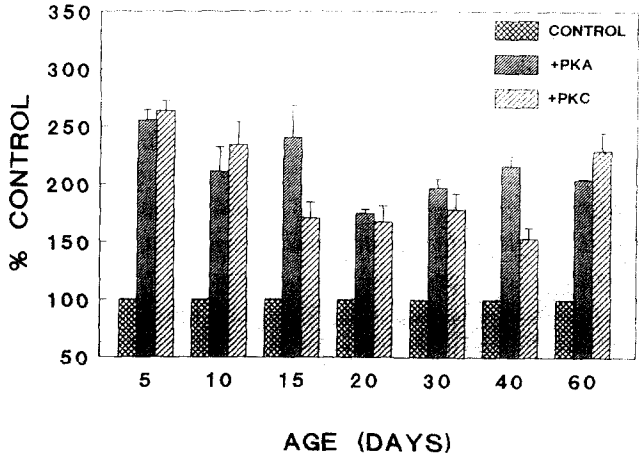

Fig. 3. Activation of CEH during development. CEH activity was measured in the absence or presence of cofactors for PKA or PKC. For activation by PKA, CEH was assayed in the presence of 1 mM $MgCl_2$, 5 mM ATP and 100 μM cAMP. For activation by PKC, the cofactors included in the assay were 1 mM $MgCl_2$, 5 mM ATP, 5 mM $CaCl_2$, 20 μg/ml phosphatidylserine and 4 μg/ml diolein. 100% control represents the specific activity obtained in the absence of cofactors. Four animals were used for each age group and values shown are mean ± S.E.M.

there was a 2.4-fold increase between 10 and 30 days, the period of active myelination. Although activity increased as a function of brain weight, specific activity ($nmol \cdot h^{-1} \cdot protein^{-1}$) did not change significantly during this period of development due to a corresponding increase in total protein (data not shown). These observations are in contrast to that reported by Eto and Suzuki[7] who observed a 2-fold increase in the specific activity of the enzyme during the period of active myelination. Between 30 and 40 days, the activity remained unchanged, whereas there was a 25% decrease in activity by 60 days during the postmyelination period, similar to the decrease in specific activity observed by Eto and Suzuki.

CEH activity was measured in the presence of cofactors for PKA and PKC as a function of age (Fig. 3). Exogenous cofactors were added to eliminate differences arising due to changes in the endogenous levels of these cofactors during development. Protein kinase-dependent activation of CEH was observed throughout development, although these studies did not distinguish between the two kinases. The finding of major interest is the correlation of this activation with the period of active myelination. Significantly higher activation ($P < 0.001$) is observed prior to the onset of myelination (5 days), suggesting that the enzyme is not present in its fully activated form and can therefore be activated substantially by the addition of exogenous cofactors. On the other hand, lower activation observed during the period of active myelination (20 days) is indicative of the presence of the enzyme in its active form. Whereas high levels of both PKA and PKC have been demonstrated in postnatal rat brain[10,19] changes observed in activation are probably not due to variations in the amount of kinases present. Between 30 and 60 days, the postmyelination period, the level of activation remained unchanged ($P > 0.05$). Our results indicate that regulation of CEH is tightly controlled and related to myelination. This finding is of importance in relation to demyelinating disorders where an increase in cholesterol ester accumulation and decrease in CEH activity are the most prominent biochemical abnormalities[22,23].

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