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Potentiation of serotonin N-acetyltransferase activity by protein kinase A and C involves two specific phosphorylation sites.

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Gene expression and protein levels of serotonin N-acetyltransferase (AA-NAT, EC2.3.1.87) directly control the diurnal production of the melatonin and correlate with enzyme activity. Effects of protein phosphorylation on the AA-NAT activity were investigated in rat pineal glands and COS-7 cells transiently transfected with AA-NAT cDNA. When the glands and transfected COS-7 cells were treated with forskolin and phorbol 12-myristate 13-acetate (PMA), AA-NAT activity and protein levels increased. These effects of forskolin and PMA could be reversed by the presence of PKA inhibitor H89 and PKC inhibitor GF109203X, respectively, suggesting that phosphorylation by protein kinase A (PKA) and protein kinase C (PKC) is involved in the potentiation of the AA-NAT activity. Cycloheximide and actinomycin D did not prevent the enhancement of the AA-NAT activity and protein levels induced by forskolin and PMA. Under such conditions, the rate of AA-NAT inactivation was reduced in the presence of forskolin, suggesting that the stability as well as the activity of AA-NAT may be regulated by protein phosphorylation. The conserved threonine (Thr) and serine (Ser) residues in AA-NAT were targeted for site-directed mutagenesis as potential sites for the phosphorylation of PKA and PKC. Mutation of Thr-29 and Ser-203 prevented the potentiation of the AA-NAT activity and protein level by forskolin and PMA in the continued presence of actinomycin D. Mutation of Thr-127 and Ser-192 was not apparently effective in the PMA effect, however, it slightly prevented potentiation of the AA-NAT activity and their protein level by forskolin. Our results, therefore, suggest that phosphorylation of AA-NAT at Thr-29, Thr-127, Ser-192, and Ser-203 by PKA or PKC is involved in either the enzymatic activity or stability of AA-NAT and that phosphorylation of Thr-29 and Ser-203 play a more important role in this AA-NAT activity or stability.