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Involvement of Raf-1 in chronic δ-opioid receptor agonist-mediated adenylyl cyclase superactivation

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Abstract

Chronic δ-opioid receptor agonist treatment of Chinese hamster ovary (CHO) cells stably expressing the human δ-opioid receptor (hDOR/CHO) leads to increased cAMP formation after the removal of the agonist (adenylyl cyclase superactivation). We have previously found that at the same time, chronic δ-opioid receptor agonist treatment augments phosphorylation of the adenylyl cyclase VI isoenzyme. Since phosphorylation of adenylyl cyclase VI by Raf-1 protein kinase was recently shown, we tested the role of Raf-1 in adenylyl cyclase superactivation in hDOR/CHO cells. We found that pretreatment of the cells with the selective Raf-1 inhibitor GW5074 (3-(3,5-dibromo-4-hydroxybenzylidene-5-iodo-1,3-dihydro-indol-2-one) (10 μM, 30 min) attenuates chronic deltorphin II-mediated increase in forskolin-stimulated cAMP formation by 40% ($n = 6$, $P < 0.05$). Better understanding of the molecular mechanism of adenylyl cyclase superactivation should aid in the development of analgesics that act longer and have fewer side effects.

Keywords: Adenylyl cyclase superactivation; δ-Opioid receptor, human; Raf-1

Chronic opioid receptor activation frequently leads to a compensatory increase in adenylyl cyclase activity (adenylyl cyclase superactivation) that becomes apparent upon agonist withdrawal as increased cAMP formation (cAMP overshoot) in response to different stimulators of adenylyl cyclase. Adenylyl cyclase superactivation after chronic opioid receptor agonist exposure is thought to play an important role in opioid tolerance, dependence and withdrawal (Williams et al., 2001).

We have previously demonstrated that chronic δ-opioid receptor agonist ((+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC 80), 1 μM, >4 h) treatment of Chinese hamster ovary (CHO) cells stably expressing the human δ-opioid receptor (hDOR/CHO) leads to adenylyl cyclase superactivation (Rubenzik et al., 2001). We have also found that chronic SNC 80 treatment augments the phosphorylation of one of the native cellular isoenzymes, adenylyl cyclase VI, in the hDOR/CHO cells (Varga et al., 1999). Phosphorylation of adenylyl cyclase VI was SNC 80 dose and treatment time-dependent and was antagonized by naltrindole (1 μM, Varga et al., 1999). The protein kinase that phosphorylates adenylyl cyclase VI in response to chronic δ-opioid receptor activation, however, has not been previously identified.

Recently, Tan et al. (2001) have found that Raf-1 protein kinase directly phosphorylates adenylyl cyclase VI. Importantly, Raf-1-mediated phosphorylation of adenylyl cyclase VI led to functional sensitization of the catalytic activity of the enzyme in response to different stimulators. Therefore, in the present work, we tested whether phosphorylation of adenylyl cyclase VI by Raf-1 is involved in chronic δ-opioid receptor agonist-mediated adenylyl cyclase superactivation in hDOR/CHO cells. We found that indeed, a selective inhibitor of Raf-1, GW5074 (3-(3,5-dibromo-4-hydroxybenzylidene-5-iodo-1,3-dihydro-indol-2-one, Lackey et al., 2000), attenuates chronic deltorphin II treatment-mediated adenylyl cyclase superactivation in hDOR/CHO cells.

hDOR/CHO cells were preincubated for 30 min (37 °C) in Iscove's modified Dulbecco's medium (IMDM) in the

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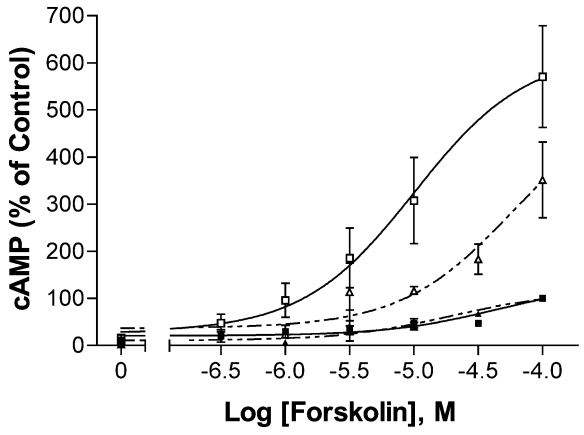

Fig. 1. The effect of the Raf-1 inhibitor, GW5074, on deltorphin II-mediated adenylyl cyclase superactivation in hDOR/CHO cells. hDOR/CHO cells were pretreated in IMDM (37 °C) in the presence (▲,△) or absence (■,□) of 10 μM GW5074 for 30 min, and subsequently incubated with (□,△) or without (■,▲) 100 nM deltorphin II for 4 h at 37 °C. The cells were thoroughly washed and forskolin dose–response curves were determined. The results (mean ± S.E.M.) are expressed as % of forskolin-stimulated cAMP formation in IMDM-treated cells in the absence of GW5074 (100%). The difference between forskolin (100 μM)-stimulated cAMP formation in hDOR/CHO cells chronically treated with deltorphin II in the presence or absence of GW5074 was significant ($n=6$, $P<0.05$).

presence or absence of the Raf-1 inhibitor, GW5074 (10 μM). After pretreatment, δ-opioid receptor agonist (deltorphin II, 100 nM) was added and the incubation continued for another 4 h. Control cells were incubated in IMDM only, in the presence or absence of GW5074. The cells were thoroughly washed and dose–response curves were determined for forskolin-stimulated cAMP formation using minor modifications of the method of Gilman (1970), as previously described (Rubenzik et al., 2001). Chronic (4 h) deltorphin II treatment augmented forskolin (100 μM)-stimulated cAMP formation to 570 ± 286% of IMDM-treated control ($n=7$) in hDOR/CHO cells (Fig. 1). Pretreatment (30 min, 37 °C) of the cells with 10 μM GW5074 attenuated chronic deltorphin II-mediated increase in forskolin (100 μM)-stimulated cAMP formation by 40% (351 ± 197% of control, $n=6$) (Fig. 1). The difference between deltorphin II-mediated cAMP overshoot in the presence and absence of GW5074 was significant ($P<0.05$).

The pathways leading to opioid receptor-mediated activation of Raf-1 are not completely understood. Raf-1 is the key protein kinase in the mitogen-activated protein kinase signal transduction cascade. The involvement of calmodulin and protein kinase C isoenzymes has been demonstrated in the pathways that led to G protein-coupled receptor-mediated activation of the mitogen-activated protein kinase cascade (Della Rocca et al., 1997). Interestingly, our preliminary data indicate that chelerythrine-sensitive isoforms of protein kinase C and calmodulin are also involved in the pathway leading to chronic δ-opioid receptor agonist-mediated phosphorylation of adenylyl cyclase VI (Varga et al., 1999, INRC abstracts, Saratoga Springs, NY).

In summary, we found that a selective inhibitor of Raf-1, GW5074, attenuates chronic δ-opioid receptor agonist-mediated adenylyl cyclase superactivation in hDOR/CHO cells. Further investigations are in progress to identify which protein kinase isoforms are regulated by the δ-opioid receptor in hDOR/CHO cells and to determine their role in the multistep process leading to Raf-1 activation, adenylyl cyclase VI phosphorylation and superactivation. Adenylyl cyclase superactivation upon chronic opioid receptor stimulation is an important molecular mechanism contributing to the development of opioid tolerance. Better understanding of the molecular events involved in drug tolerance upon chronic δ-opioid receptor treatment should aid in the development of analgesics that act longer and have fewer side effects.

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References

Della Rocca, G.J., van Biesen, T., Daaka, Y., Lutrell, D.K., Lutrell, L.M., Lefkowitz, R.J., 1997. Ras-dependent mitogen activated protein kinase activation by G protein coupled receptors: convergence of Gi- and Gq-mediated pathways on calcium/calmodulin, Pyk2 and Src kinase. J. Biol. Chem. 272, 19125–19132.

Gilman, A.G., 1970. A protein binding assay for adenosine 3′5′-cyclic monophosphate. Proc. Natl. Acad. Sci. U. S. A. 67, 305–312.

Lackey, K., Cory, M., Davis, R., Frye, S.V., Harris, P.A., Hunter, R.N., Jung, D.K., McDonald, O.B., McNutt, R.W., Peel, M.R., Rutkowske, R.D., Veal, J.M., Wood, E.R., 2000. The discovery of potent cRaf1 kinase inhibitors. Bioorg. Med. Chem. Lett. 10, 223–226.

Rubenzik, M., Varga, E., Stropova, D., Roeske, W.R., Yamamura, H.I., 2001. Expression of alpha-transducin in Chinese hamster ovary cells stably transfected with the human delta-opioid receptor attenuates chronic opioid agonist-induced adenylyl cyclase superactivation. Mol. Pharmacol. 60, 1076–1082.

Tan, C.M., Kelvin, D.J., Litchfield, D.W., Ferguson, S.S.G., Feldman, R.D., 2001. Tyrosine kinase-mediated serine phosphorylation of adenylyl cyclase. Biochem. 40, 1702–1709.

Varga, E.V., Stropova, D., Rubenzik, M., Waite, S., Roeske, W.R., Yamamura, H.I., 1999. Phosphorylation of adenylyl cyclase VI upon chronic δ-opioid receptor stimulation. Eur. J. Pharmacol. 364, R1–R2.

Williams, J.T., Christie, M.J., Manzoni, O., 2001. Cellular and synaptic adaptations mediating opioid dependence. Physiol. Rev. 81, 299–343.