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SENSITIVITY CHANGES OF ENTERIC CHOLINERGIC NEURONS TO μ- AND κ-OPIOID RECEPTOR AGONISTS AFTER CHRONIC SYMPATHETIC DENERVATION: ROLE OF PROTEIN KINASE C

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Different inhibitory receptor pathways, including α_2-adrenoceptors, μ- and κ-opioid receptors, are involved in the modulation of the cholinergic function in the guinea-pig distal colon. At this level, chronic sympathetic denervation entails development of supersensitivity to the inhibitory effect of μ- and κ-opioid receptor agonists as well as subsensitivity to the inhibitory effect α_2-adrenoceptor agonists on acetylcholine (ACh) release (1,2). On the whole, these observations are highly suggestive for the existence of a functional interplay between different inhibitory inputs on the enteric cholinergic function.

The molecular mechanisms underlying sensitivity changes of the enteric cholinergic function to inhibitory agents after chronic sympathetic denervation are still unknown. In the present study we evaluated the possible involvement of protein kinase C (PKC) in the development of supersensitivity to opioid receptor agonists in this experimental condition. In a first series of experiments we investigated whether activation of PKC might influence ACh overflow from the myenteric plexus of the guinea-pig distal colon. To this end, the effect of phorbol-12-miristate-13-acetate (PMA) on spontaneous endogenous ACh overflow was studied in the absence and presence of PKC inhibitors, calphostin and cheleritrin. In addition, we evaluated the effect of DAMGO (μ-opioid receptor agonist) and U69593 (κ-opioid receptor agonist) on ACh overflow in the presence of the phospholipase C inhibitor, U73122 and of calphostin and cheleritrin. Spontaneous endogenous ACh overflow was measured from isolated specimens superfused with Tyrode's solution. The superfusate was assayed for ACh by HPLC-ED. The effect of sympathetic denervation on PKC expression has been evaluated in synaptosomal preparations of myenteric neurons, by immunoblot assay.

PMA dose-dependently enhanced spontaneous endogenous ACh overflow from the guinea-pig colon. The maximal effect was obtained at 1 μM and was significantly lower in preparations obtained from sympathetically-denervated animals than in preparations obtained from normal animals. The effect of 1 μM PMA was significantly reduced by calphostin (100 nM) and cheleritrin (1 μM) in preparations obtained both from normal and sympathetically-denervated animals. Calphostin (100 nM) and cheleritrin (1 μM) per se did not modify ACh overflow.

U69593 (100 nM) and DAMGO (100 nM) inhibited ACh overflow from normal preparations. 500 nM U73122, 100 nM calphostin and 1 μM cheleritrin significantly increased the inhibitory effect of both agonists. In preparations obtained from sympathetically-denervated animals, the inhibitory effect of U69593 and DAMGO on ACh overflow was significantly higher than in those obtained from normal animals and was not modified by PLC and PKC inhibitors.

Western blot analysis of PKC revealed one band at 85 kDa. The intensity of the immunoreactive band corresponding to PKC was lower in myenteric synaptosomes obtained from sympathetically-denervated animals than in those obtained from normal animals.

The present data indicate that activation of PKC enhances spontaneous ACh release in the myenteric plexus of the guinea-pig colon. However, the inability of PKC inhibitors to influence ACh overflow would exclude a tonic PKC-mediated facilitation of the neurotransmitter release, at this level. After chronic sympathetic denervation, subsensitivity to the facilitatory effect of PMA on ACh overflow as well as the lower expression levels of PKC are indicative of a reduced efficiency of this signalling pathway in this experimental condition. In our model, enhancement of DAMGO and U69593 inhibitory effects on ACh overflow in the presence of PKC inhibitors, might suggest that μ- and κ-opioid pathways activate PKC, which in turns attenuates opioid-mediated inhibition of ACh overflow. The ability of the PLC inhibitor, U73122, to enhance DAMGO and U69593 effects indicates that both μ- and κ-opioid pathways modulate PKC in enteric cholinergic neurons via PLC. After chronic sympathetic denervation, PLC and PKC inhibitors did not modify the effect of both DAMGO and U69593. This data together with the reduced efficiency of PKC raise the question whether this signalling pathway contributes to the development of supersensitivity to the inhibitory effect of opioid agonists in this experimental condition.

References

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