TITLE: OPIOID TOLERANCE—EFFECT OF PERTUSSIS TOXIN
AUTHORS: C.S. Wong, MD, K.J. Chang, PhD, Y.F. Su, PhD, and W.D. Watkins, MD, PhD
AFFILIATION: Dept of Anes, Duke Univ Med Ctr, Durham, N.C. and Burroughs Wellcome Co, RTP, N.C.

Guanine nucleotide regulatory proteins (G-proteins) are involved in a wide range of cellular signaling mechanisms. The ability of G-proteins to transmit signals initiated at the receptor level is disrupted following ADP-ribosylation by pertussis toxin.[1] Opioid receptors are G-protein linked receptors. Prolonged opiate exposure causes tolerance, the mechanism of which is unknown. Pertussis toxin (i.c.v.) abolishes the analgesic effect of morphine,[2] suggesting that uncoupling of the opioid receptor from G-proteins might be one of the mechanisms of opioid tolerance.

Double intrathecal (i.t.) catheterized male Sprague-Dawley rats were prepared for i.t. injection of all drugs used in this study. Analgesia to opioid was evaluated by the tail flick test.[3] In the treated group, rats were injected i.t. with pertussis toxin (1μg/10 μl). Control rats received an equal volume of saline. The basal threshold of tail flick latency and the analgesic effect of μ-agonist PL017 (i.t., 0.5 μg/rat) were tested in both groups at 1, 3, 7 and 14 days after injection of pertussis toxin. Tests of analgesia were performed 30 min after PLO17 was administrated acutely. After the analgesic test, rats were sacrificed and the spinal cords removed for opioid receptor binding assays. ^{125}I-FK33824 (μ-agonist) and ^{3}H-CTOP (μ-antagonist) were used as radiolabeled ligands to characterize the opioid receptor binding properties and the guanine nucleotide regulatory effect on opioid receptor binding in both naive and toxin-treated animals.

The i.t. injection of a single dose (1μg/rat) of pertussis toxin did not induce apparent behavioral deficits. The toxin began to affect the PLO17 induced analgesia 1 day after toxin administration and lasted for 14 days. The ^{125}I-FK33824 binding assay showed that the high affinity state μ-opioid receptor decreased by 70% in the treated animals. The ^{3}H-CTOP binding assays showed that the agonist competition binding curve was shifted to the right by pertussis toxin treatment, but the Bmax was not affected.

These results are consistent with the concept that pertussis toxin treatment uncouples opioid receptors from G-proteins, and shifts opioid receptors from a high affinity state to a low affinity state. This alteration of opioid affinity state correlates with a decrease in the opioid analgesic effect. Thus, our study suggests that receptor uncoupling is a significant mechanism of opioid tolerance.

References:
1. J. Biol. Chem. 1981, 256:8310.
2. Eur. J. Pharmacol. 1986, 124:357.
3. J. Pharmacol. Exp. Ther. 1987, 240:150.

A717

TITLE: REGULATION OF ENDOTHELIN-STIMULATED CALCIUM RELEASE FROM INTRACELLULAR STORES IN VASCULAR SMOOTH MUSCLE CELLS BY ISOPROTERENOL
AUTHORS: Yu-Ting Xuan, A Richard Whorton, and W David Watkins
AFFILIATION: Depts of Anesthesiology and Pharmacology Duke University Medical Center Durham, North Carolina 27710

Endothelin (ET) is a 21 amino acid peptide synthesized primarily by the vascular endothelium. This peptide is a potent vasoconstrictor which causes slow, tonic contraction of vascular smooth muscle. We have shown that the mechanism of action of ET on smooth muscle cells involves production of $Ins(1,4,5)P_3$, mobilization of intracellular Ca^{2+} and Ca^{2+} influx through a voltage-sensitive Ca^{2+} channel. In *in vivo* studies both the Ca^{2+} channel blocker, nicardipine, and the β-adrenergic receptor agonist, isoproterenol (ISO) inhibit ET-induced contraction. However, the mechanism is not clear.

In previous studies, we demonstrated that the inhibitory effect of nicardipine was due to blockade of Ca^{2+} influx stimulated by ET. We have also found that ISO increases production of cAMP and inhibits ET-mediated formation of $Ins(1,4,5)P_3$. To further understand the regulation of ISO on ET-induced vasoconstriction, we have investigated the effects of ISO on ET-induced Ca^{2+} mobilization both in intact and saponin-permeabilized vascular smooth muscle cells.

Determination of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) was performed by the fura-2 methods. When intact cells were perfused with Ca^{2+}-complete buffer and stimulated by 10 nM ET for 1 min, $[Ca^{2+}]_i$ rose rapidly from a basal value of 50 ± 8 nM to a peak of 370 ± 24 nM within 1 min followed by a sustained rise. This response represented both release of intracellular Ca^{2+} and influx of extracellular Ca^{2+}. When cells were perfused with Ca^{2+}-free buffer containing 1 mM EGTA, ET induced a transient rise in $[Ca^{2+}]_i$. The peak was only 195 ± 20 nM and retured to basal levels within 2 min. This represented Ca^{2+} release from internal stores only. When cells were perfused with Ca^{2+}-complete buffer containing 1 μM ISO and then challenged with 10 nM ET, both the peak change in $[Ca^{2+}]_i$ and the duration of this response were attenuated by 49% and 58% respectively.

To investigate whether ISO regulates Ca^{2+} release from intracellular stores, cells were perfused with Ca^{2+}-free buffer containing 1 mM EGTA with or without 1 μM ISO. ET-induced Ca^{2+} release stimulated by ET in ISO treated cells was inhibited by 36% when compared to control. This effect was reversed by β-receptor antagonist, propranolol, and was mimicked by the direct agonist of adenylate cyclase, forskolin. This suggests that a cAMP-dependent mechanism might be involved.

In saponin-permeabilized cells, addition of $Ins(1,4,5)P_3$ induced a dose-dependent release of Ca^{2+} from non-mitochondrial stores. Treatment of permeabilized cells with cAMP resulted in an inhibition of $Ins(1,4,5)P_3$-induced Ca^{2+} release from these stores and shifted the dose-response curve of $Ins(1,4,5)P_3$-induced Ca^{2+} release to the right. Finally, intact cells were first pretreated with ISO or forskolin and then permeabilized. The response to $Ins(1,4,5)P_3$ was also inhibited by 39% following ISO and 38% following forskolin pretreatment.

We conclude that ISO regulates ET-induced Ca^{2+} release from intracellular stores by a direct inhibitory effect on $Ins(1,4,5)P_3$-induced Ca^{2+} release from internal stores. This data suggests an important interaction between the two major second messenger systems