

TITLE: OPIOID TOLERANCE—EFFECT OF PERTUSSIS TOXIN
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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 (1 μ g/10 μ l). Control rats received an equal volume of saline. The basal threshold of tail flick latency and the analgesic effect of μ -agonist PLO17 (i.t., 0.5 μ g/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 administered acutely. After the analgesic test, rats were sacrificed and the spinal cords removed for opioid receptor binding assays. ¹²⁵I-FK33824 (μ -agonist) and ³H-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.

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TITLE: REGULATION OF ENDOTHELIN-STIMULATED CALCIUM RELEASE FROM INTRACELLULAR STORES IN VASCULAR SMOOTH MUSCLE CELLS BY ISOPROTERENOL
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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)P₃, mobilization of intracellular Ca²⁺ and Ca²⁺ influx through a voltage-sensitive Ca²⁺ channel. In *in vivo* studies both the Ca²⁺ channel blocker, nifedipine, 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 nifedipine was due to blockade of Ca²⁺ influx stimulated by ET. We have also found that ISO increases production of cAMP and inhibits ET-mediated formation of Ins(1,4,5)P₃. To further understand the regulation of ISO on ET-induced vasoconstriction, we have investigated the effects of ISO on ET-induced Ca²⁺ mobilization both in intact and saponin-permeabilized vascular smooth muscle cells.

Determination of intracellular Ca²⁺ concentrations ([Ca²⁺]_i) was performed by the fura-2 methods. When intact cells were perfused with Ca²⁺-complete buffer and stimulated by 10 nM ET for 1 min, [Ca²⁺]_i rose rapidly from a basal value of 50 \pm 8 nM to a

peak of 370 \pm 24 nM within 1 min followed by a sustained rise. This response represented both release of intracellular Ca²⁺ and influx of extracellular Ca²⁺. When cells were perfused with Ca²⁺-free buffer containing 1 mM EGTA, ET induced a transient rise in [Ca²⁺]_i. The peak was only 195 \pm 20 nM and returned to basal levels within 2 min. This represented Ca²⁺ release from internal stores only. When cells were perfused with Ca²⁺-complete buffer containing 1 μ M ISO and then challenged with 10 nM ET, both the peak change in [Ca²⁺]_i and the duration of this response were attenuated by 49% and 58% respectively.

To investigate whether ISO regulates Ca²⁺ release from intracellular stores, cells were perfused with Ca²⁺-free buffer containing 1 mM EGTA with or without 1 μ M ISO. ET-induced Ca²⁺ 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.

References:

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

In saponin-permeabilized cells, addition of Ins(1,4,5)P₃ induced a dose-dependent release of Ca²⁺ from non-mitochondrial stores. Treatment of permeabilized cells with cAMP resulted in an inhibition of Ins(1,4,5)P₃-induced Ca²⁺ release from these stores and shifted the dose-response curve of Ins(1,4,5)P₃-induced Ca²⁺ release to the right. Finally, intact cells were first pretreated with ISO or forskolin and then permeabilized. The response to Ins(1,4,5)P₃ was also inhibited by 39% following ISO and 38% following forskolin pretreatment.

We conclude that ISO regulates ET-induced Ca²⁺ release from intracellular stores by a direct inhibitory effect on Ins(1,4,5)P₃-induced Ca²⁺ release from internal stores. This data suggests an important interaction between the two major second messenger systems