TITLE: INHIBITION BY ISOPROTERENOL OF ENDOTHELIN-MEDIATED PRODUCTION OF INOSITOL PHOSPHATES IN VASCULAR SMOOTH MUSCLE CELLS

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Altered vascular reactivity and tone are important features of many disease states. Although the exact nature of the mediators involved is not known, recent evidence suggests that vasoconstrictor substances produced by the endothelium play a pivotal role. Of particular importance is the recently discovered vasoactive peptide, endothelin (ET). ET is extremely potent in constricting vascular smooth muscle from a variety of vascular beds. In intact vascular preparations, the actions of ET can be partially blocked by voltage-dependent Ca^{2+} channel antagonists and a β-adrenergic receptor agonist, isoproterenol (ISO). It is important to understand not only the cellular mechanism for ET-induced contraction of vascular smooth muscle but also to understand the regulation of these responses by these drugs.

In previous studies, we have demonstrated that ET may induce contraction by stimulating the production of inositol phosphates and mobilizing both Ca^{2+} release from intracellular stores and influx of Ca^{2+} from outside the cell through a voltage-dependent or L-type Ca^{2+} channel at the plasma membrane (1). In order to understand the mechanism for ISO inhibition of ET-induced contraction of smooth muscle cells, we have used A10 vascular smooth muscle cells to investigate the effects of ISO on ET-induced production of inositol phosphates.

Cells were labelled with [^{3}H] inositol for 24 hours and treated with 10 nM ET for 1 minute. ET significantly stimulates the production of InsP, Ins(1,4)P_2, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 reaching about 2-fold above control for Ins(1,4)P_2 and Ins(1,4,5)P_3 measured by HPLC. When cells were pretreated with ISO for 10 minutes and then stimulated by the same dose of ET, the production of each isomer of inositol phosphates was reduced. However, ISO did not alter the basal levels of inositol phosphates. In addition, when inositol phosphates were measured after 10 minutes of incubation with increasing concentrations of ET, ISO was found to inhibit the sustained formation of total inositol phosphates. In these experiments it appears that the maximum level of stimulation was decreased while the EC_{50} was not altered. This suggests that ISO may regulate ET-induced inositol phosphate formation by a mechanism beyond the receptor.

To investigate this possibility, we have further investigated the effect of forskolin, a direct agonist of adenylate cyclase, on ET-induced inostiol phosphate formation. It was found that forskolin mimicked the inhibitory effect of ISO on ET-stimulated inositol phosphate formation. Finally, when cells were treated with ISO or forskolin, the production in intracellular cAMP increased by 14-fold in ISO-treated and 11-fold in forskolin-treated cells when compared to control cells. The effect of ISO was totally reversed by the non-selective β-adrenergic receptor antagonist, propranolol. These results support the idea that the inhibitory effect of ISO on ET-mediated inositol phosphate formation may be regulated by a cAMP-dependent mechanism.

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A745

Title: Memory Evanescence In Mice Exposed To Halothane
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Introduction: Inhalational anesthetics affect memory. Anterograde amnesia has been demonstrated with halothane using an animal model of inhibitory passive avoidance learning.[1] This study examines the amnestic effects of halothane as a function of time.

Method: After institutional approval, 80 adult Swiss-Webster mice (25-35 gm) were studied. Prior to training, half were exposed to air (A) and half to halothane (H). Mice were anesthetized for 30 mins in a 5L chamber into which 2% halothane in O_2 flowed at 6 L/min. Soda lime was placed underneath a perforated plate in the chamber. Training of H mice began 15 mins after return of ambulation. A and H were trained in a two-compartment (dark and illuminated) shuttle chamber. Each mouse was placed in the illuminated side and a door opened, allowing passage to the dark side where a 0.2 mA shock was administered for 1.0 sec. Initial training latencies were compared between A and H to assess adequate locomotor recovery. A and H, divided into 4 subgroups, were tested in the shuttle chamber at 1, 3, 7 and 10 days after training (n=10). Memory of shock retention was ascertained by measuring crossover time from illuminated to dark side (testing latency) with maximal score of 300 sec. Data analyzed using two-way ANOVA and Student-Newman Keuls test.

Results: No significant difference occurred between training latencies of A and H. Results are summarized in Fig 1. H test latencies were significantly less than A at all times. There was a significant effect of the time of testing where test day 3 latency was greater than any other day within the H group ($p<.05$).

Discussion: Exposure to halothane had an amnestic effect on day 1 probably due to retrieval failure. This is supported by the improved memory seen on day 3. Shock memory declined on days 7 and 10. Scopolamine has a similar qualitative temporal decrement on memory retention.[2] In undrugged rats, memory improvement with time has been demonstrated using active avoidance learning.[3] It is possible that after normal learning, increasing sensitivity to neurotransmitters occurs at specific synapses. With time, sensitivity declines and leads to forgetting.[4] Halothane exposure prior to training may interfere with memory consolidation and produce a weakened memory trace which temporarily improves as a result of the transiently increased synaptic sensitivity, then declines as this effect wanes.

References:
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Fig 1. Test latencies when mice are exposed to air or halothane prior to training