Title: EFFECTS OF PERTUSSIS TOXIN ON MORPHINE-HALOTHANE INTERACTION IN THE GUINEA PIG ILEUM.

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It has been demonstrated that the effects of many hormones and transmitters are mediated by a group of guanine nucleotide-binding proteins (G or N) located within cell membranes. At present, four types of G proteins have been identified (Gs, Gi, Gt, Go); they modulate adenylate cyclase activity, cGMP phosphodiesterase, phospholipase C and possibly calcium and potassium ion channels. Gs and Gi are substrates for cholera and pertussis toxins (PT) respectively; thus treatment with the toxins has been used to study the involvement of G proteins in a given system. In the guinea pig ileum, both opiates and halothane inhibit the muscle contractions induced by electrical stimulation. The present study investigates the consequences of pretreatment with PT on the effect of morphine (MS) and halothane (H) as well as on their interaction.

Methods: We have used the electrically stimulated myenteric plexus-longitudinal muscle preparation (MPLM) from the guinea pig ileum. Two groups of animals were injected i.p. with saline or PT (60 ug/kg in saline) and sacrificed the fifth day after injection. The following groups of experiments were performed in saline treated animals: Dose response curves to MS alone, H alone and MS in the presence of H (1.6 V/V%). An identical series of experiments was performed in PT treated animals. The slopes of the individual curves and the IC_{50}'s of MS and H under different experimental conditions were determined and compared. An analysis of the interaction was performed by the method of Chou and Chou utilizing experiments in which H and MS were used in fixed ratios.

Results and Discussion: In the saline group, MS and H each produced dose-related inhibitions of the electrically induced muscle contractions with IC_{50}'s of 1.9 x 10^{-7}M and 1.7 V/V% respectively. In animals pretreated with PT the potency of H was significantly decreased. Table 1 shows that the effect of PT varied at different concentrations of H. Thus, the concentration of H needed to produce a 16% inhibition was unaltered by the toxin while those needed to produce a 50 and 84% inhibition were significantly elevated ($p<0.05$). In animals treated with PT, the IC_{50} of MS was unchanged (Table 2). When dose response curves to MS were obtained in the presence of 1.6% H, the potency of MS was significantly increased ($p<0.05$) in the saline group. Pretreatment with PT abolished this effect, returning the IC_{50} of MS to its original value in the absence of H. The effect of PT on MS-H interaction also varied at different levels of response. Thus, the IC_{16} of MS in the presence of H was unaltered by the toxin, while the IC_{50} and IC_{84} were significantly increased. When the combined effects of MS and H were analyzed, the interaction was found to be less than additive at low levels of response and synergistic at high levels. PT altered the interaction such that it became less than additive at all levels of response.

TABLE 1
EFFECT OF PERTUSSIS TOXIN ON THE POTENCY OF HALOTHANE

	Halothane V/V%		
Group	IC_{16} (CL)	IC_{50} (CL)	IC_{84} (CL)
Saline	0.71 (0.5-1.0)	1.7 (1.4-1.9)	12.0 (5.8-21)
Pertussis	1.1 (0.9-1.4)	8.7* (4.8-15.9)	67.8* (25-105)

* $p<0.05$ between pretreatment groups.

TABLE 2
EFFECT OF PERTUSSIS TOXIN ON MORPHINE-HALOTHANE INTERACTION

	Pretreatment			
	Saline		Pertussis	
	no H	1.6% H	no H	1.6% H
MS IC_{50} x10^{-7}M	1.9	0.67*	2.1	1.7
CL	(1.6-2.1)	(.5-.8)	(1.7-2.7)	(.9-3.4)

* $p<0.05$ when compared to each of the other values.

Our results demonstrate that: 1) The effect of halothane and 2) the increased potency of morphine in the presence of halothane are antagonized by pretreatment with pertussis toxin. This strongly suggests that the mechanism of action of halothane, but not that of morphine, is mediated by the substrate for pertussis toxin, presumably a Gi membrane protein present in the guinea pig ileum.