Opioid dependence prevents the action of pertussis toxin in the guinea-pig myenteric plexus

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Summary. The longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum has been employed for the study of the effect of pertussis toxin (IAP) on opioid dependence. Guinea-pigs were treated with IAP (120 µg/kg, i.p.) either prior to chronic administration of an opioid or after opioid dependence had been established. The isolated preparations were tested in vitro for dependence; that is, the naloxone-precipitated withdrawal contracture. Naloxone almost failed to evoke a sign of dependence in preparations treated with IAP prior to chronic exposure to an opioid. In contrast, IAP failed to affect the withdrawal contracture when applied to an animal after dependence has been established. It is concluded that the N1-unit, the substrate for IAP, plays a critical function in the development of dependence. The continuous activation of the opioid receptor associated with the development of dependence may induce changes in N1 which in turn prevent the interaction of IAP with its substrate.

Key words: Opioid dependence — Pertussis toxin — Guinea-pig myenteric plexus

Introduction
Adenylate cyclase has been suggested to mediate inhibitory actions of opiates in the central nervous system (Collier and Roy 1974) and neuronal hybrid cells (Sharma et al. 1975). More recent evidence in support of this concept originates from work with pertussis toxin (islet activating protein, IAP). This neurotoxin has been demonstrated to bind highly selectively to the GTP-binding protein (N1), a component of the multifactorial adenylate cyclase system (Ui 1984). Employing pertussis vaccine or IAP itself, Collier et al. (1983) and Tucker (1984) observed an attenuation of the naloxone-precipitated opioid withdrawal contracture (that is, dependence; Schulz 1978) of isolated segments of the guinea-pig ileum rendered dependent either in vitro or in vivo. From these experiments it was concluded that the adenylate cyclase may play a pivotal role in the development of opioid dependence in the myenteric plexus (Tucker 1984).

The present investigation examines more closely the effect of IAP on the development of opioid dependence in the guinea-pig myenteric plexus. It has been reported that the interaction between IAP and the N1-unit may be decreased during the activated state of the N1-linked receptor system (Tsai et al. 1984; Van Dop et al. 1984). Since continuous stimulation of the opioid receptor is a prerequisite for the development of dependence, we hypothesized that IAP should fail to block the development of dependence when the opioid-controlled N1-unit is continuously activated.

Methods
Male guinea pigs (300 g) were decapitated and the ileum dissected. The longitudinal muscle-myenteric plexus (strip) was set up in a 5 ml organ bath in Krebs-Ringer-bicarbonate solution (37°C for electrical field stimulation (60 V, 0.5 ms, 0.1 Hz; Schulz and Goldstein 1973), and equilibrated for 1 h. These electrical parameters evoked maximal twitch tension (range 2.5—3.0 g).

Chronic administration of an opioid was carried out by subcutaneous implantation of osmotic minipumps (model 2001, Alza Corp., Palo Alto, CA, USA). The pump delivered 1 µg fentanyl per hour (1 µg/ml water). In order to maintain dependence of the strips in vitro, the preparations were incubated with the opioid infused in vivo. The fentanyl concentration selected was 5 nM which failed to depress electrically evoked twitches and yielded maximal signs of dependence. For assessment of dependence, the strips were challenged with naloxone (100 nM) in the absence of electrical stimulation (Schulz and Herz 1976). The intensities of the withdrawal contractures precipitated are expressed as a percentage of the preceding electrically-evoked twitch tension.

The effect of IAP was tested by a single injection (i.p.) of the neurotoxin. The animals received the toxin in 0.5 ml saline. For the schedule of treatment with fentanyl and IAP, see Results.

Statistical analysis: the values are expressed as means ± SEM. Significance of differences was calculated by use of the Student's two-tailed t-test.

The following substances were used: fentanyl dihydrogencitrate (Janssen, Beerse, Belgium), pertussis toxin (IAP; List Biol. Labs, Campbell, CA, USA), naloxone-HCl (Endo Labs, Garden City, NJ, USA).

Results
In previous experiments with the isolated guinea-pig ileum a maximal effect of IAP was observed 3 to 6 days following a single injection (Lujan et al. 1984; Tucker 1984). To examine...
drawal contracture, the guinea pigs received a single injection of 20, 60 and 120 µg/kg of the toxin either 3 or 6 days prior to sacrifice. Subsequently, 2 days prior to decapitation the animals were infused with fentanyl (1 µg/h). Figure 1 demonstrates a similar effect of pretreatment with IAP at 3 or 6 days on the intensity of the withdrawal contracture. The IAP dosage required to obtain 90% inhibition was 120 µg/kg. Sixty µg/kg reduced the withdrawal sign by about half.

Figure 2 illustrates the experimental conditions required for a modification of dependence in the myenteric plexus by IAP (120 µg/kg). Panel A reveals that IAP given 3 days prior to the fentanyl infusion strongly reduces the naloxone-precipitated withdrawal contracture. The intensity of the withdrawal contracture was not affected when fentanyl was infused 3 days prior to the toxin (panel B). However, upon application of the fentanyl infusion only 12 h prior to IAP (3 days), the toxin was still able to attenuate the withdrawal contracture to a considerable extent (panel C). Again, the sign of dependence was maximally reduced when IAP was given 24 h prior to fentanyl (panel D). The intensity of withdrawal in strips taken from animals exposed for 6 days only to fentanyl (1 µg/h) was not different from those infused for 2 days (1 µg/h fentanyl), and amounted to 60% of electrically-evoked twitches.

Discussion

The major finding reported here is the inability of IAP to interfere with dependence when applied subsequent to its development. On the other hand, IAP does prevent the development of dependence when given prior to chronic exposure to an opioid which confirms previous findings by Tucker (1984).

Since development and maintenance of dependence require the continuous occupation (activation) of opioid receptors (Schulz and Herz 1976), the data may be in favour of the concept that IAP fails to interfere with the N1-regulatory protein during the state of receptors activation. Only the inactivated system is sensitive to the neurotoxin. Analogous data have been reported for the effect of IAP on transducin, a component of the retinal photon receptor protein rhodopsin (Tsai et al. 1984; Van Dop et al. 1984). However, such mechanism does not sufficiently explain the present findings. Panel C of Fig. 2 illustrates that despite an activation of the opioid receptor 12 h prior to IAP exposure, the toxin still interferes to a considerable degree with the development of dependence. Apparently, the degree of dependence induced prior to IAP exposure may be of significance. There are presently no data regarding the nature of the adaptation which occurs within 12 h of opioid exposure. Although this rather short period can produce alterations in the myenteric plexus resembling dependence (Schulz 1978; Collier et al. 1983), 3 days of exposure to an opioid is known to cause profound changes, such as the induction of neuronal supersensitivity (Schulz and Goldstein 1973; Schulz and Herz 1976).