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Pertussis Toxin Pretreatment Affects Opiate/Nonopiate and Stress-Induced Analgesia Differently

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PAROLARO, D., P. MASSI, G. PATRINI, T. RUBINO, M. PARENTI AND E. GORI. *Pertussis toxin pretreatment affects opiate/nonopiate and stress-induced analgesia differently.* PHARMACOL BIOCHEM BEHAV **38**(3) 569–573, 1991.—Intracerebroventricular injection of pertussis toxin (PTX, 1 μg/rat) six days before the hot plate test abolished analgesia induced by central morphine. The toxin did not affect analgesia evoked by central neurotensin or ASU 1–7 eel calcitonin. PTX pretreatment also attenuated footshock-induced analgesia (FSIA) delivered to all four paws. When the shock was restricted to the front paws, PTX consistently lowered postshock tail flick latencies, but did not reduce analgesia resulting from shock delivered to the hind paws. It thus appears that PTX-sensitive G-proteins are an essential transduction step needed to initiate the molecular events underlying opiate analgesia evoked by either morphine or shock. In contrast, the signal transduction mechanism subsequent to the stimulation of neurotensin or calcitonin receptors, and to the nonopiate FSIA, appears not to involve PTX-sensitive G-proteins.

Pertussis toxin Analgesia ASU 1–7 eel calcitonin Neurotensin Foot shock-induced analgesia

IN the last few years several studies have shown that pertussis toxin (PTX)-sensitive G-proteins are involved in the antinociception induced by stimulation of opioid receptors (10,13). Intracerebroventricular (ICV) pretreatment of rats with PTX significantly reduces the analgesic effect of morphine, regardless of the route of opioid administration (ICV, into the periaqueductal gray matter, intrathecally or intraperitoneally) or the analgesic test adopted (tail flick, jaw opening reflex) (11). The effect of PTX is dose-dependent, noncompetitive and does not appear to be equivalent for different opioid ligands since the blockade produced by the toxin can be overcome by higher doses of μ ligand (DAGO) but not by comparable high doses of δ ligand (DADLE) (13). Despite these results on opioid peptides, data about the involvement of G-protein-dependent mechanisms in nonopioid and stress-induced analgesia are lacking.

Therefore, in the present study, we tested in rats the effectiveness of PTX pretreatment in antagonizing two distinct forms of analgesia: 1) induced by centrally administered nonopioid peptides such as ASU 1–7 eel calcitonin (ASU-CT) and neurotensin (NT), and 2) antinociception elicited by continuous electrical footshock.

METHOD

Animals

Male Sprague-Dawley rats, supplied by Charles River (Calco, Italy), weighing 180–200 g were used, fed a pellet diet with water ad lib. Environmental conditions were standardized (22 ± 2°C, 60% humidity and 12 h artificial lighting per day).

Intracerebroventricular Microinjections

Rats were anesthetized with tribromoethanol (200 mg/kg IP) and prepared for ICV microinjections according to Altaffer's procedure (1). Briefly, anesthetized rats were fixed in a stereotaxic apparatus and the right lateral ventricle was located using a stereotaxic atlas (12). A permanent polyethylene cannula (Ulrich and Co., type PE10) was implanted so as to penetrate the ventricle 4.5 mm from the top of the skull, to which it was then fixed with dental cement (Hottinger Baldwin Messtechnick, type X60). After the operation the animals were placed in individual cages and allowed to recover for five days.

Drug solutions in a constant volume of 5 μl were injected into conscious rats by inserting a Hamilton microsyringe into the cut

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cannula tip. Control rats were injected ICV with 5 μl of saline. At the end of each experiment the rats were killed by decapitation and 5 μl of Evans blue dye (0.5%) was injected through the cannulas. After 5 min the brains were removed and placed in 10% formalin. They were frozen 24 h later, cut to a thickness of 80 μm and examined microscopically to verify cannula placements.

Analgesic Assays

The analgesic effects were assessed using the tail flick method or the hot plate test.

The tail flick test was performed according to D'Amour and Smith (5) in which a beam of light is focused on the dorsal side of the tail and the latency to flick the tail away from the noxious stimulus is measured. A cut-off of 8 s was used to prevent tissue damage and the rat was removed from the apparatus if it failed to respond within this interval. The results for each time were expressed as latency in seconds and the total area under the time-response curve (AUC) was calculated as long as the analgesic effect lasted.

For the hot plate test a hot plate apparatus (IITC, model 35D, supplied by Basile, Comerio, Varese, Italy) with accurate thermostatic control was employed. Rats were placed on the heated (58°C) surface of this apparatus and the paw reaction (rapid lifting and licking) was used as the end point. A 30-s cut-off time was used to avoid blistering. The results for each time were expressed as latency in seconds and the total area under the time-response curve (AUC) was calculated.

Footshock Analgesia

Footshock analgesia was evoked according to the procedure of Watkins et al. (16), summarized here. Before exposure to footshock each rat was tested for baseline pain responsiveness using the tail flick test. This procedure consisted of three tail flick trials (TF), separated by 2-min intertrial intervals (ITI). The results of these trials were averaged to give a mean baseline latency (BL, in seconds) for each rat. After this baseline procedure, the rats were exposed once to an electrical shock delivered, depending on the experiment, to either the front paws, the hind paws or all four paws. To limit the shock exposure to a restricted body region, a soft rubber cord was gently looped around either the abdomen or chest of the rat and then pulled up so that only the front or hind paws, as needed, were in contact with the shock grid. Each rat was placed in a Plexiglas box with a grid floor and was exposed to 90 s of 1.6 mA electrical shock. At the end of the footshock, tail flick trials were used to assess changes in pain responsiveness up to 14 min postshock. The stimulus was automatically terminated at 8 s if no tail flick occurred, in order to avoid tissue damage.

Drugs

The following drugs were used: pertussis toxin (List Biological Labs, Campbell, CA, dissolved in 0.05 M sodium phosphate buffer pH 7 with 0.15 M NaCl); morphine hydrochloride (S.I.F.A.C., Milan, Italy); Asu 1–7 eel calcitonin (kindly supplied by I.S.F., Trezzano sul Naviglio, Milan, Italy); neurotensin (Sigma Chemical Co., St. Louis, MO); naloxone hydrochloride (S.I.F.A.C., Milan, Italy).

Statistical Analysis

One-way analysis of variance (ANOVA) was done by collapsing data across all groups. This was followed by individual group comparisons using Tukey's test (15).

RESULTS

The effect of treating rats with PTX (1 μg/rat ICV) six days before evaluating the antinociceptive effect of morphine (M), Asu 1–7 eel calcitonin (ASU-CT) and neurotensin (NT) is illustrated in Fig. 1. Basal nociceptive thresholds were the same in pretreated rats and controls. As expected, in the hot plate test, ICV microinjections of M, ASU-CT and NT evoked significant analgesia lasting respectively 90, 120 and 60 min. In the PTX-pretreated rats M had significantly less antinociceptive effect, the time-response curve being reduced by 60%. Pretreatment with ICV PTX only partially reduced the antinociception elicited by ICV NT, the area under the time-response curve for PTX-pretreated rats being about 20% less than for NT alone (Fig. 1A).

PTX pretreatment did not affect the analgesia induced by ASU-CT. In fact, throughout the entire time course, the analgesia evoked by this peptide was the same in controls and PTX-pretreated rats. However, it must be noted that all PTX-pretreated rats showed diffuse body tremor immediately after ASU-CT microinjection, ending in a convulsive syndrome in 38% of rats. These signs persisted for 120 min, sometimes making a proper assessment of analgesia difficult.

Figure 2 shows that the shock delivered to all four paws reliably induced a powerful antinociceptive response (tail flick test) throughout the remainder of the 14-min postshock period. The foot shock-induced analgesia (FSIA) was greatly attenuated in the PTX-pretreated rats (Fig. 2) and the area under the time-response curve (AUC) for PTX-pretreated rats was 32% less than for FSIA alone. The analgesia resulting from shock restricted to the hind paws or to the front paws is illustrated in Fig. 3. As previously demonstrated (16), the antinociception resulting from shock to the front paws was significantly antagonized by naloxone pretreatment; however, the hind paws analgesia was not reversed by naloxone so indicating that the body region shocked is one determinant of the opiate or nonopiate nature of FSIA. Figure 3 also shows the effect of PTX pretreatment on opiate and nonopiate FSIA. When the shock was delivered to the front paws (opiate analgesia), PTX pretreatment consistently lowered postshock tail flick latencies and the area under the curve was significantly reduced, by about 30%. PTX had no effect on analgesia resulting from shock only to the hind paws.

DISCUSSION

The present study provides evidence of different roles of PTX-sensitive G-proteins in the mediation of opiate/nonopiate and stress-induced analgesia. Antinociception elicited by M was significantly antagonized by PTX pretreatment while analgesia evoked by central-acting NT or ASU-CT was not affected. Thus it appears that PTX-sensitive G-proteins are not responsible for the signal transduction mechanism subsequent to the stimulation of NT and ASU-CT receptors. NT stimulates the release of inositol phosphate in rat brain slices and in murine neuroblastoma clone N1E-115 (7,14), but does not affect either basal or stimulated cAMP levels. G-proteins are involved in the receptor (NT) stimulated breakdown of phosphoinositides although the identity of this/these G-proteins is still unknown, mainly because of the differing effects of PTX. In some systems, receptor-mediated stimulation of phospholipase C activity is attenuated by treatment with the toxin; in others PTX has no effect (2).

This latter observation raises the possibility of there being other types of G-protein that do not serve as substrate for PTX ribosylation. Recently, Bozou et al. (3) reported the existence of both

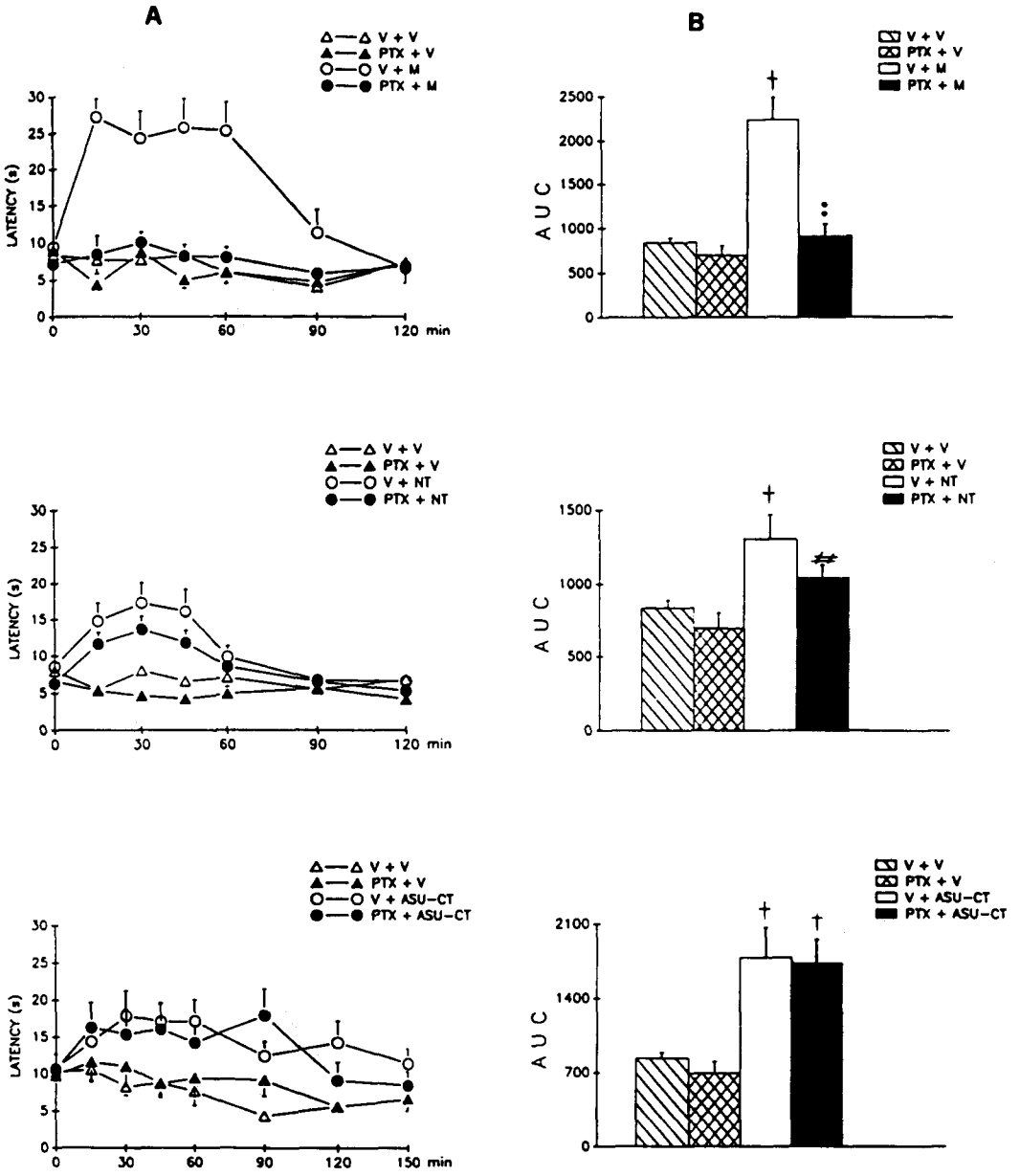

FIG. 1. (A) Effect of ICV PTX (1 μg/rat, 6 days before) on analgesia (hot plate test) induced by ICV morphine (M, 5 μg/rat), neurotensin (NT, 50 μg/rat) and ASU 1–7 eel calcitonin (ASU-CT, 1.25 μg/rat). V = vehicle (controls). (B) Effect of PTX pretreatment expressed as area under time-response curve (AUC), see A. †$p<0.01$ vs. control group (Tukey's test). **$p<0.01$ vs. M alone (Tukey's test). #$p<0.05$ vs. NT alone (Tukey's test).

PTX-sensitive and insensitive mechanisms of NT-mediated inhibition of cAMP formation in the N1E-115 cell line. It thus appears that the G-proteins involved in NT analgesia (Gi and/or Gp) are insensitive to PTX. However, PTX pretreatment slightly reduced NT analgesia (about 20%) and this partial reduction could be due to ADP ribosylation of PTX-sensitive Gi; alternatively, this reduction could be ascribed to the action of the toxin on G-proteins associated with other modulators involved in the analgesic effect of NT. PTX had no effect on analgesia evoked by ASU-CT, although there is evidence that calcitonins of different origin do have inhibitory effects on adenylate cyclase in different brain areas (8). In other systems the peptide stimulates rather than inhibits the enzyme (4, 9, 18). Finally, there is some evidence that calcium ions are involved in the central effects of calcitonins (6). One can, therefore, conclude that there must be different classes of calcitonin receptors that can be coupled to adenylate cyclase (for either stimulation or inhibition) and/or to calcium ions. In our hands, PTX did not affect ASU-CT analgesia, suggesting that G-proteins coupled to adenylate cyclase are not involved in the analgesia. Curiously, ASU-CT treatment in rats given PTX six days before evoked a diffuse body tremor which sometimes ended in convulsive behavior. This picture was probably associated with the PTX pretreatment. By inactivating the influence of some G-protein inhibitory-dependent mechanism

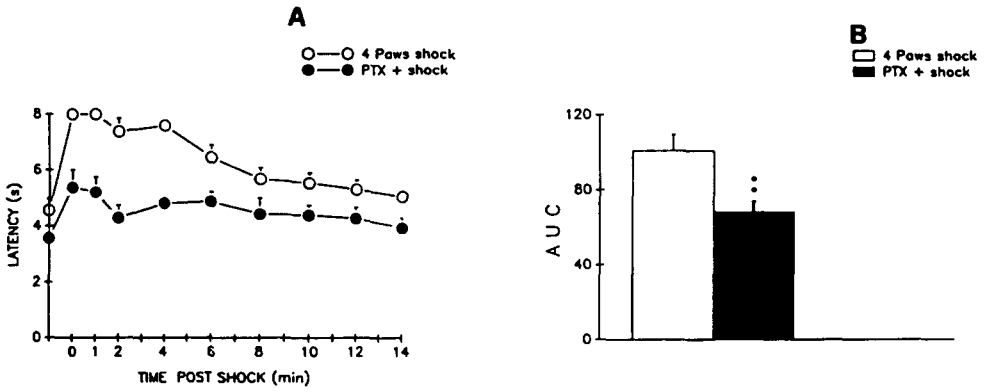

FIG. 2. (A) Effect of ICV PTX (1 μg/rat, 6 days before) on analgesia (tail flick test) induced by shock delivered to all four paws. (B) Effect of PTX pretreatment expressed as area under time-response curve (AUC), see A. $^{**}p<0.01$ vs. vehicle + four paws shock (Student's t-test).

PTX may very well cause behavioral excitation.

Finally, our findings on stress-induced analgesia indicate that G-proteins have different effects on opiate and nonopiate forms of FSIA. The body region shocked appears to be at least one critical factor in determining the opiate or nonopiate nature of FSIA which involves at least three pain modulatory systems (17). The first two pathways, which mediate the nonopiate analgesia evoked by hind-paw shock, consist of an intraspinal system and a descending dorsolateral funiculus (DLF) pathway with supraspinal origin. The third pathway, of the neural opiate analgesia evoked by front-paw shock, acts through a descending path within the DLF and depends on an opiate synapsis within the spinal cord (17). Included as neural opiate analgesia are also stimulation-produced analgesia and morphine analgesia following systemic, intracerebral or intrathecal administration. Our data indicate that PTX-sensitive G-proteins are an essential transduction step in initiating the molecular events underlying neural/opiate analgesia activated through either morphine or shock. In contrast, nonopi-

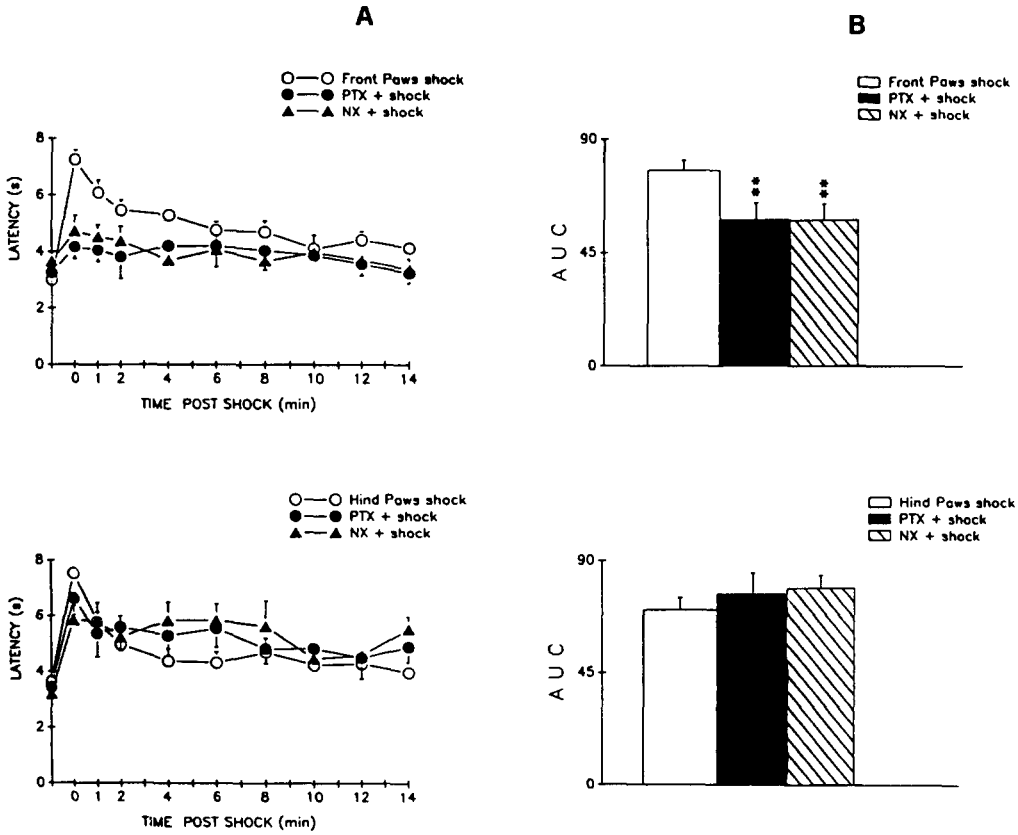

FIG. 3. (A) Effect of ICV PTX (1 μg/rat, 6 days before) or naloxone (Nx, 10 mg/kg IP ×2) on analgesia (tail flick test) induced by shock delivered to front or hind paws. (B) Effect of PTX or Nx pretreatment expressed as area under time-response curve (AUC), see A. ** $p<0.01$ vs. vehicle + front paws shock (Tukey's test).

ate FSIA was not affected by PTX pretreatment, suggesting that it could be linked to neural PTX-insensitive G-proteins coupled to different effector systems (e.g., ion channels, phosphoinositides breakdown, etc.).

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