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BRAIN RESEARCH

Research Report

Pre-treatment with a PKC or PKA inhibitor prevents the development of morphine tolerance but not physical dependence in mice

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ABSTRACT

We previously demonstrated that intracerebroventricular (i.c.v.) administration of protein kinase C (PKC) or protein kinase A (PKA) inhibitors reversed morphine antinociceptive tolerance in 3-day morphine-pelleted mice. The present study aimed at evaluating whether pre-treating mice with a PKC or PKA inhibitor prior to pellet implantation would prevent the development of morphine tolerance and physical dependence. Antinociception was assessed using the warm-water tail immersion test and physical dependence was evaluated by quantifying/scoring naloxone-precipitated withdrawal signs. While drug-naïve mice pelleted with a 75 mg morphine pellet for 3 days developed a 5.8-fold tolerance to morphine antinociception, mice pre-treated i.c.v. with the PKC inhibitors bisindolylmaleimide I, Go-7874 or Go-6976, or with the myristoylated PKA inhibitor, PKI-(14-22)-amide failed to develop any tolerance to morphine antinociception. Experiments were also conducted to determine whether morphine-pelleted mice were physically dependent when pre-treated with PKC or PKA inhibitors. The same inhibitor doses that prevented morphine tolerance were evaluated in other mice injected s.c. with naloxone and tested for precipitated withdrawal. The pre-treatment with PKC or PKA inhibitors failed to attenuate or block the signs of morphine withdrawal including jumping, wet-dog shakes, rearing, forepaw tremor, increased locomotion, grooming, diarrhea, tachypnea and ptosis. These data suggest that elevations in the activity of PKC and PKA in the brain are critical to the development of morphine tolerance. However, it appears that tolerance can be dissociated from physical dependence, indicating a role for PKC and PKA to affect antinociception but not those signs mediated through the complex physiological processes of withdrawal.

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Abbreviations: PKC, protein kinase C; PKA, protein kinase A; ED_{50}, 50% effective dose; %MPE, percent maximum possible effect; i.c.v., intracerebroventricular; s.c., subcutaneous; Bis, (2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide hydrochloride; Go-7874, Go-7874 12-(2-Cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c)-carbazole; Go-6976, 12-(2-Cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c) carbazole; PKI-(14-22)-amide, Myr-N-Gly-Arg-Thr-Gly-Arg-Arg-Asn-Ala-Ile-NH_2

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1. Introduction

Opioids such as morphine are the most widely used drugs for the alleviation of moderate to severe chronic pain. Systemically administered morphine produces antinociception via actions at both spinal and supra-spinal sites (Barton et al., 1980). Morphine activates descending systems within the brainstem that inhibit dorsal horn nociceptive neurons (Basbaum and Fields, 1984) as well as directly inhibiting spinal cord neurons to prevent transmission to supra-spinal centers (Yaksh and Noueihed, 1985).

However, repeated use of opioids induces tolerance that results in the loss of drug effect or requires escalating doses to produce pain relief. The neurobiological mechanisms of the development of opioid tolerance are multifaceted and only partially understood. Important mechanisms involved in opioid tolerance are cellular and molecular adaptation processes like receptor uncoupling from G-protein (desensitization), opioid agonist-induced receptor

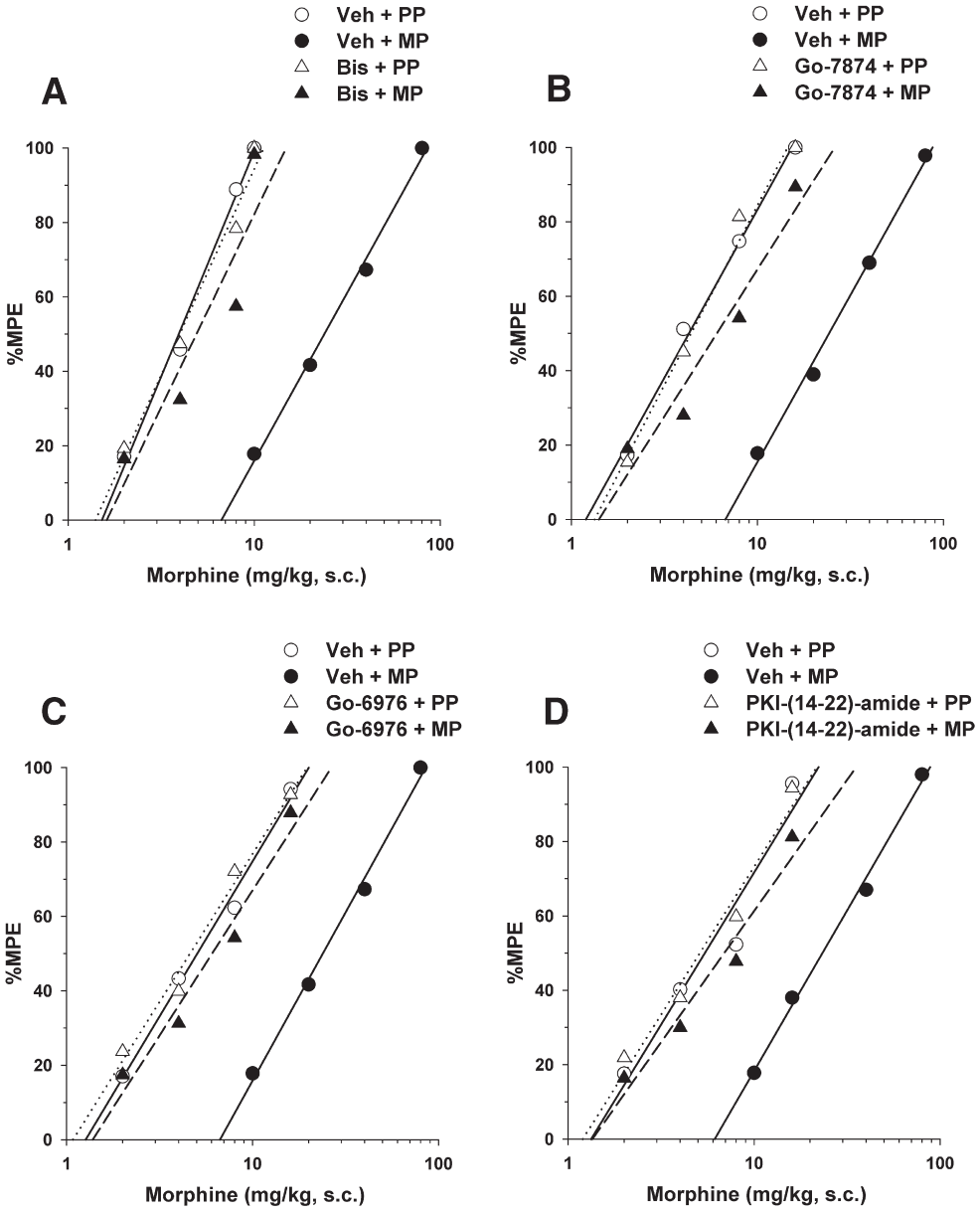

Fig. 1 – Prevention of morphine antinociceptive tolerance in mice by pre-treatment with bisindolylmaleimide I (A), Go-7874 (B), Go-6976 (C) or PKI-(14-22)-amide (D). Mice were injected i.c.v. with vehicle (Veh), bisindolylmaleimide I (Bis; 44.4 nmol/mouse), Go-7874 (15 nmol/mouse) or Go-6976 (25 nmol/mouse) 1 h prior to placebo pellets (PP) or 75 mg morphine pellets (MP). PKI-(14-22)-amide was administered i.c.v. 1 h prior to pellet implantation and at 24 and 48 h following pellet implantation. At 72 h following implantation mice were challenged with various doses of morphine s.c. and their tail immersion latencies were determined for construction of dose–response curves. Data are expressed as mean % MPE ± S.E.M. Each data point represents 6–10 mice.