Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 773–776, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0145-8

BRIEF COMMUNICATION

Spinal and Supraspinal Effects of Pertussis Toxin on Opioid Analgesia

SUKRUT SHAH, ALOKESH DUTTAROY, TRONG DAVIS AND BYRON C. YOBURN[1]

Department of Pharmaceutical Sciences, College of Pharmacy, St. John's University, Queens, NY 11439

Received 13 December 1993

SHAH, S., A. DUTTAROY, T. DAVIS AND B. C. YOBURN. *Spinal and supraspinal effects of pertussis toxin on opioid analgesia.* PHARMACOL BIOCHEM BEHAV **49**(3) 773–776, 1994.—The effects of in vivo pertussis toxin (PTX) treatment on the functional effects of opioid agonists were examined in the mouse. Mice were injected intracerebroventricularly (ICV), or intrathecally (IT), or IT and ICV with PTX, and dose-response studies of the antinociceptive action of systemic (SC) morphine, fentanyl, and etorphine were conducted 10 days later. IT PTX decreased the potency (≈4.5-fold) of morphine more than ICV administration (≈1.5-fold), whereas the combination of IT and ICV administration produced an additive effect. When PTX was administered spinally and supraspinally, the potency of morphine, fentanyl, and etorphine was reduced similarly (≈5–7-fold), indicating that the effect of PTX does not vary considerably among agonists of different intrinsic efficacies. These studies indicate that in vivo PTX can reduce the potency of opioid agonists with different intrinsic efficacies, and that spinal mechanisms appear to be more sensitive to PTX treatment.

Pertussis toxin Opioid analgesia Opioid receptors Spinal analgesia Supraspinal analgesia Morphine Fentanyl Etorphine

THE ROLE that second messengers play in the effects of opioids has been widely investigated. Studies indicate that pertussis toxin (PTX)-sensitive mechanisms are involved in binding–effector coupling in the opioid system. In in vivo systems PTX treatment can limit analgesic and other effects of opioids in both rats and mice (3,8,15,16,18,20,22,25). Similarly, in vitro studies have shown that opioid actions are blocked or inhibited by prior PTX treatment (1,4,5,7,9,12,13,21,23). The effect of PTX has been suggested to involve the ADP-ribosylation of the inhibitory variety of the guanine nucleotide (G_i) proteins, as well as G_o, which is another G-protein believed to be involved in ion flux [e.g., (10,19)]. These coupling elements (G_i, G_o) are presumably linked to a variety of intracellular effectors, including adenylyl cyclase, outward K^+ flux, and regulation of Ca^{++} currents [e.g., (10)]. Regardless of the exact intracellular events that couple opioid binding to effect, it is clear that many of these effects are PTX sensitive. Therefore, PTX can be used as a probe to help uncover the cellular mechanisms of opioid actions.

Studies of the effect of in vivo PTX on the systemic analgesic potency of opioids have been limited to morphine. Compounds that differ in intrinsic efficacy have not been examined. Thus, in the present studies, the in vivo effect of PTX on the antinociceptive potency of three opioid agonists (morphine, fentanyl, and etorphine) that differ in intrinsic efficacy (2,11,17) was compared. In addition, we have examined PTX-sensitive spinal and supraspinal mechanisms in morphine analgesia.

METHOD

Subjects

Male Swiss Webster mice (Taconic Farms, Germantown, NY) were used throughout (23–40 g). Mice were housed 5–10 per cage with free access to food and water.

[1] Requests for reprints should be addressed to Byron C. Yoburn, Ph.D., College of Pharmacy and Allied Health Professions, St. John's University, 8000 Utopia Parkway, Queens, NY 11439.

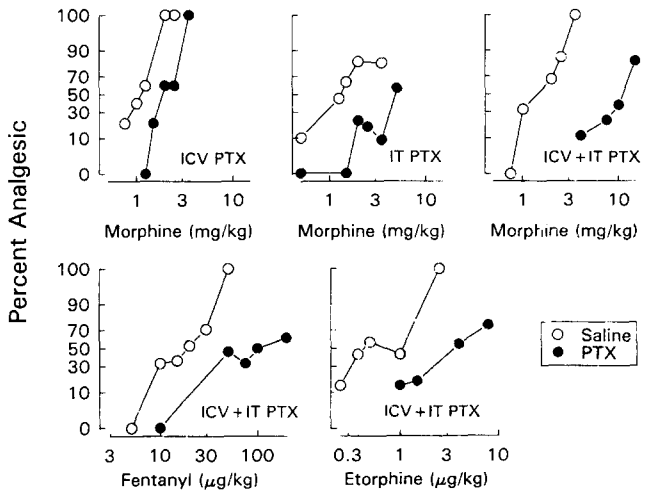

FIG. 1. The effect of ICV, IT, and ICV + IT pertussis toxin on morphine, fentanyl, and etorphine analgesia. Mice were injected ICV (top left panel), IT (top center panel), or ICV + IT (top right panel and bottom panels) with saline or PTX (0.1 μg per site). Total dose was 0.1, 0.1, and 0.2 μg per mouse, respectively. Ten days later mice were injected (SC) with morphine (top panels), fentanyl (bottom left panel), or etorphine (bottom right panel) and tested for analgesia. Representative results are shown. To facilitate comparisons, the relative lengths of the x-axes are constant for each panel. See Table 1.

Procedure

Mice were injected intracerebroventricularly (ICV) and/or intrathecally (IT) with PTX (0.1 μg per site). PTX was administered ICV (5 μl) and IT (2 μl) while mice were lightly anesthetized with halothane : oxygen (4 : 96). Ten days later mice were tested in dose-response studies using the tail flick assay (see below).

Antinociception Assay

Ten days following PTX treatment, mice were weighed, and a baseline tail flick was determined. The tail flick apparatus was adjusted so that baseline flicks were typically between 2-4 s. Mice were then injected SC with morphine (0.5-15.0 mg/kg; n = 6-7/dose per treatment), fentanyl (5.0-200 μg/kg; n = 7/dose per treatment), or etorphine (0.25-10 μg/kg; n = 7/dose per treatment) and tested for antinociception (analgesia) using the tail flick 30 min (morphine) or 15 min (etorphine, fentanyl) later. A cutoff of 10 s was used to avoid tissue damage. Mice that did not flick by 10 s were defined as analgesic.

Drugs

Morphine sulfate was donated by Penick Laboratories (Newark, NJ). PTX was obtained from List Biological Laboratories (Campbell, CA). Fentanyl citrate was purchased from Sigma Chemical (St. Louis, MO). Etorphine HCl was obtained from the Research Technology Branch of the National Institute on Drug Abuse. Drugs were dissolved in 0.9% NaCl and doses are expressed as the base.

Data Analysis

Dose-response data were analyzed by Probit Analysis (6) using a computerized program (BLISS 21, Department of Statistics, University of Edinburgh) that estimates ED_{50}s, 95% confidence limits, and relative potencies.

RESULTS

Baseline nociceptive sensitivity was not altered 10 days following PTX treatment. The baseline tail flick latencies for

TABLE 1

THE EFFECT OF ICV, IT, AND ICV + IT PERTUSSIS TOXIN ON OPIOID ANALGESIA

Drug and Treatment	Saline (ED_{50})	PTX (ED_{50})	Relative Potency	Shift
Morphine (mg/kg)				
ICV PTX	1.55 (1.31-1.80)	2.57* (2.08-3.11)	0.60	1.66
IT PTX	1.24 (0.86-1.81)	5.95* (3.28-13.53)	0.21	4.80
IT + ICV PTX	1.49 (1.09-1.98)	10.23* (7.79-14.31)	0.15	6.87
Fentanyl (μg/kg)				
IT + ICV PTX	19.97 (12.58-42.18)	101.76* (59.22-209.10)	0.20	5.10
Etorphine (μg/kg)				
IT + ICV PTX	0.61 (0.34-1.15)	3.66* (2.00-7.47)	0.17	6.00

Mice were injected ICV, IT, or ICV + IT with saline or PTX (0.1 μg per site). Total dose was 0.1, 0.1, and 0.2 μg per mouse, respectively. Ten days later mice were tested for morphine, fentanyl, or etorphine (SC) analgesia. Data presented are ED_{50}s (95% confidence limits) from a representative study. Relative potency is the ED_{50} for saline divided by the ED_{50} for PTX. The shift is the ED_{50} for PTX divided by the ED_{50} for saline. Each study was conducted two-three times.

*Significantly different from corresponding saline-treated group: $p < 0.01$.

PTX-treated mice and controls did not differ significantly [2.12 ± 0.16 s (SD), 2.28 ± 0.47; saline, PTX-treated, respectively). PTX shifted the dose-response function for morphine analgesia to the right following ICV (Fig. 1, top left), IT (Fig. 1, top center), and ICV and IT (Fig. 1, top right) administration. ED_{50}s, relative potencies, and potency shifts are summarized in Table 1. IT PTX produced a greater effect on morphine analgesia than ICV PTX. The combined action of IT and ICV PTX was approximately additive. In a separate study, a higher dose of ICV PTX (0.2 μg) did not further decrease morphine potency [morphine ED_{50}s (95% confidence limits) = 1.06 mg/kg (0.86–1.30), 2.05* (1.70–2.49), 2.04* (1.66–2.45); saline, 0.1 μg ICV PTX, 0.2 μg ICV PTX, respectively; *significantly different from saline-treated, $p < 0.01$].

Combined IT and ICV PTX shifted the ED_{50} for etorphine (Fig. 1, bottom left) and fentanyl (Fig. 1, bottom right) comparably (Table 1). The shifts in the ED_{50}s for fentanyl and etorphine were roughly similar to that observed for morphine following IT and ICV PTX.

DISCUSSION

These results are consistent with and extend previous reports that in vivo PTX can inhibit the effects of systemic morphine [e.g., (3,16)]. The present study demonstrates that in vivo IT and ICV PTX can reduce the analgesic potency of three systemically administered opioid agonists to produce analgesia in the mouse. IT plus ICV PTX was effective in reducing the potency of all three opioid agonists (morphine, etorphine, and fentanyl) to a similar degree. Thus, the difference in intrinsic efficacy did not appear to alter sensitivity to PTX. It might have been predicted that high intrinsic efficacy compounds such as etorphine couple more efficiently with G-proteins and thus the shift would have been substantially less than that observed for morphine. However, the PTX-induced reduction in functional G-proteins similarly affected the potency of all three agonists. Comparable results have been reported for locally (ICV) administered morphine and etorphine in PTX-treated mice (20), although in that study the effect of PTX on several ICV-administered selective μ and δ agonists is reduced somewhat more than ICV morphine.

The 0.1-μg dose of PTX was more effective in reducing morphine potency when administered IT compared to ICV administration. This finding may represent enhanced sensitivity of the cord, or the fact that the mass of the cord is less than the brain, thus resulting in higher spinal cord concentrations of PTX. However, we found that higher ICV doses of PTX (0.2 μg) did not increase the shift of the morphine dose-response curve to the right. This suggests that the effect of PTX is maximal at 0.1 μg. This finding agrees with our previous results (3) in which we found that morphine potency was not significantly different in mice treated with 0.2 or 0.5 μg PTX administered ICV and IT (total dose = 0.4 and 1.0 μg). Another possible explanation for the reduced effect of ICV PTX is that the toxin may have encountered barriers between the ventricle and more distant relevant sites of action in the brain (24). Finally, it is worth noting that PTX treatment did not alter baseline tail flick latencies. It might have been expected that if the animals were adversely affected by PTX that baseline tail flick latencies would have increased and that mice might have been more sensitive to opioid agonists. However, this was not the case and, therefore, we conclude that inhibition of opioid analgesia by PTX was not due to nonspecific effects.

In summary, in vivo PTX treatment of mice reduced similarly the systemic potency of morphine, etorphine, and fentanyl. Furthermore, spinal mechanisms appear to be more sensitive than supraspinal mechanisms to the effects of in vivo PTX.

ACKNOWLEDGEMENTS

Our thanks to Penick Laboratories for generously supplying morphine sulfate. Our thanks to Dr. M. T. Turnock for helpful discussions. These data represent a portion of a thesis for the MS degree in pharmaceutical sciences by S.S. Part of these data were presented at the 1993 meetings of the American Society for Pharmacology and Experimental Therapeutics, New Orleans, LA. This project was supported by the National Institute on Drug Abuse (DA 04185).

REFERENCES

1. Abood, M. E.; Law, P. Y.; Loh, H. H. Pertussis toxin treatment modifies opiate action in the rat brain striatum. Biochem. Biophys. Res. Commun. 127:477–483; 1985.
2. Adams, J. U.; Paronis, C. A.; Holtzman, S. G. Assessment of relative intrinsic activity of mu-opioid analgesics in vivo by using β-funaltrexamine. J. Pharmacol. Exp. Ther. 255:1027–1032; 1990.
3. Chang, S. C. J. L.; Lutfy, K.; Sierra, V.; Yoburn, B. C. Dissociation of opioid receptor upregulation and functional supersensitivity. Pharmacol. Biochem. Behav. 38:853–859; 1991.
4. Collier, H. O. J.; Plant, N. T.; Tucker, J. F. Pertussis vaccine inhibits the chronic but not the acute action of normorphine on the myentric plexus of guinea-pig ileum. Eur. J. Pharmacol. 91: 325–326; 1983.
5. Costa, T.; Aktories, K.; Schultz, G.; Wuster, M. Pertussis toxin decreases opiate receptor binding and adenylate cyclase inhibition in a neuroblastoma × glioma hybrid cell line. Life Sci. 33:219–222; 1983.
6. Finney, D. J. Probit analysis, 3rd ed. London: Cambridge University Press; 1973.
7. Gintzler, A. R.; Xu, H. Different G proteins mediate the opioid inhibition or enhancement of evoked [5-methionine]enkephalin release. Proc. Natl. Acad. Sci. USA 88:4741–4745; 1991.
8. Hoehn, K.; Reid, A.; Sawynok, J. Pertussis toxin inhibits antinociception produced by intrathecal injection of morphine, noradrenaline and baclofen. Eur. J. Pharmacol. 146:65–72; 1988.
9. Law, P. Y.; Louie, A.; Loh, H. Effect of pertussis toxin treatment on the downregulation of opiate receptors in neuroblastoma × glioma NG108-15 hybrid cells. J. Biol. Chem. 260:14818–14823; 1985.
10. Law, P. Y.; Loh, H. H. Second messengers in the transduction of multiple opioid receptor signals. In: Watson, R. R., ed. Drugs of abuse and neurobiology. Boca Raton, FL: CRC Press; 1992:39.
11. Liao, C. S.; Day, A. R.; Freer, R. J. Evidence for a single opioid receptor type on the field stimulated rat vas deferens. Life Sci. 29: 2617–2622; 1981.
12. Lujan, M.; Lopez, E.; Ramirez, R.; Aguilar, H.; Martinez-Olmedo, M. A.; Garcia-Sainz, J. A. Pertussis toxin blocks the action of morphine, norepinephrine and clonidine on isolated guinea-pig ileum. Eur. J. Pharmacol. 100:377–380; 1984.
13. Lux, B.; Schulz R. Opioid dependence prevents the action of pertussis toxin in the guinea-pig myenteric plexus. Naunyn Schmeidebergs Arch. Pharmacol. 330:184–186; 1985.
14. Lux, B.; Schulz, R. Effect of cholera toxin and pertussis toxin on opioid tolerance and dependence in the guinea-pig mysenteric plexus. J. Pharmacol. Exp. Ther. 237:995–1000; 1986.
15. Parenti, M.; Tirone, F.; Giagnoni, G.; Pecora, N.; Parolaro, D. Pertussis toxin inhibits the antinociceptive action of morphine in the rat. Eur. J. Pharmacol. 124:357–359; 1986.
16. Parolaro, D.; Patrini, G.; Giagnoni, G.; Massi, P.; Groppetti, A.; Parenti, M. Pertussis toxin inhibits morphine analgesia and

prevents opiate dependence. Pharmacol. Biochem. Behav. 35: 137–141; 1990.
17. Paronis, C. A.; Holtzman, S. G. Development of tolerance to the analgesic activity of mu agonists after continuous infusion of morphine, meperidine or fentanyl in rats. J. Pharmacol. Exp. Ther. 262:1–9; 1992.
18. Przewlocki, R.; Costa, T.; Lang, J.; Herz, A. Pertussis toxin abolishes the antinociception mediated by opioid receptors in rat spinal cord. Eur. J. Pharmacol. 144:91–95; 1987.
19. Reisine, T. Pertussis toxin in the analysis of receptor mechanisms. Biochem. Pharmacol. 39:1499–1504; 1990.
20. Sanchez-Blazquez, P.; Garzon, J. Pertussis toxin differentially reduces the efficacy of opioids to produce supraspinal analgesia in the mouse. Eur. J. Pharmacol. 152:357–361; 1988.
21. Schulz, R. Dependence and cross-dependence in the guinea-pig myenteric plexus. Naunyn Schmeidebergs Arch. Pharmacol. 337: 644–648; 1988.
22. Suzuki, T.; Funada, M.; Narita, M.; Misawa, M.; Nagase, H. Pertussis toxin abolishes μ and δ-opioid agonist-induced place preference. Eur. J. Pharmacol. 205:85–88; 1991.
23. Tucker, J. F. Effects of pertussis toxin on normorphine-dependence and on acute inhibitory effects of normorphine and clonidine in guinea-pig isolated ileum. Br. J. Pharmacol. 83:326–328; 1984.
24. van der Ploeg, I.; Cintra, A.; Altiok, N.; Askelöf, P.; Fuxe, K.; Fredholm, B. B. Limited distribution of pertussis toxin in rat brain after injection into the lateral cerebral ventricles. Neuroscience 44:205–214; 1991.
25. Wong, C.-S.; Su, Y.-F.; Chang, K.-J.; Watkins, W. D. Intrathecal pertussis toxin treatment attenuates opioid antinociception and reduces high-affinity state of opioid receptors. Anesthesiology 77:691–699; 1992.