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Rapid communication

Opioid receptors and acetaminophen (paracetamol)

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Abstract

We report that the acetaminophen (paracetamol)-induced spinal (intrathecal, i.t.)/supraspinal (intracerebroventricular, i.c.v.) site/site antinociceptive 'self-synergy' in mice is attenuated by the opioid receptor subtype selective antagonists β-funaltrexamine hydrochloride (β-FNA; μ), naltrindole (δ), and nor-binaltorphine hydrochloride (nor-BNI; κ). These findings further implicate endogenous opioids (viz., endorphins, enkephalins, and dynorphins) and their pathways as contributors to the central antinociceptive action of acetaminophen.

Keywords: Acetaminophen; Paracetamol; Opioid receptor

More than 100 years after its synthesis, the mechanism of analgesic action of acetaminophen (paracetamol; *N*-acetyl-*p*-aminophenol) remains unknown. Postulated mechanisms (reviewed by Walker, 1995 and Björkman, 1995), including inhibition of cyclooxygenase isozymes, have been inadequate (Warner et al., 2004). However, recent work in our laboratory has led to the discovery that the analgesic effect of acetaminophen involves recruitment of endogenous opioid pathways that lead to antinociceptive spinal–supraspinal 'self-synergy' (Raffa et al., 2000). We also demonstrated a synergistic enhancement of acetaminophen's antinociceptive action by spinal administration of phentolamine (Raffa et al., 2001), implicating an interaction between descending endogenous opioid pathways and spinal sites. The present study used opioid receptor subtype-selective antagonists to identify the opioid receptor subtype(s) (μ, δ, or κ) involved in the spinal/supraspinal antinociceptive synergy elicited by acetaminophen. Elucidation of the details of this contribution should enhance the understanding of the mechanism of this major analgesic pathway, with possible benefit to novel drug-design efforts.

The methods were similar to those previously reported (Raffa et al., 2000). Male pathogen-free Swiss-derived albino Crl:CD-1®(ICR)BR mice (18–24 g; Charles River Laboratories) were group-housed under controlled conditions of temperature, humidity and 12-h light/dark cycle. Food and water were available ad libitum. Each mouse was used once and was treated in accordance to the principles expressed in the Declaration of Helsinki. The standard abdominal irritant test described by Collier et al. (1968), with minor modifications, was used. Briefly, acetaminophen (45 μg) or vehicle (5% ethanol–distilled water) was injected into the right lateral cerebral ventricle (5 μl), into the subarachnoid space of the subvertebral space between L5 and L6 (5 μl), or both (22.5 μg each site). The mice were then injected with acetylcholine bromide (5.5 mg/kg., intraperitoneally (i.p.)) and observed for up to 10 min for a single characteristic behavioral response as described by Collier et al. (1968). The absence (inhibition) of this response over 10 min was calculated as % antinociception according to: 100×(nonresponders)/(group size). The opioid receptor antagonists naloxone hydrochloride (nonselective, 3.6 μg), naltrindole hydrochloride (δ-selective, 5 μg), and nor-binaltorphine hydrochloride (nor-BNI; κ-selective, 5 μg) were administered intrathecally (i.t.) together with acetaminophen or vehicle. The μ-selective β-funaltrexamine hydrochloride (β-FNA; 10 mg/kg, subcutaneously (s.c.))

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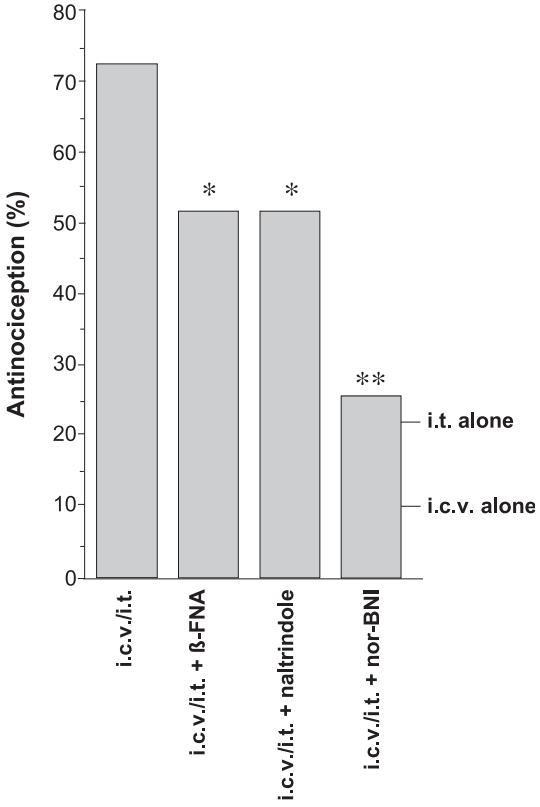

Fig. 1. Antinociception, expressed as percent of the maximum possible (total number of mice in group), following administration of acetaminophen both i.c.v. and i.t. (i.c.v./i.t.) with or without one of the subtype-selective opioid antagonists β-FNA (μ), naltrindole (δ), or nor-BNI (κ). *N*=20–51 per group. *P* value (Fisher's Exact Test for quantal data applied prior to normalization) compared to i.c.v./i.t. administration (**P*<0.05; ***P*<0.01).

was administered 24 h prior to acetaminophen or vehicle. Statistical significance (*P*<0.05 criterion) was assessed using two-sided Fisher's Exact Test.

Acetaminophen produced antinociception by either intracerebroventricular (i.c.v.) or i.t. routes alone and site/site synergistic effect when the same total dose was administered both i.c.v. and i.t. (*P*<0.005 compared to i.c.v. alone; *P*<0.001 compared to i.t. alone; Fig. 1). Naloxone attenuated the site/site synergy, reducing acetaminophen's antinociceptive effect toward that of single-site administration (not significantly different, *P*>0.05, from i.c.v. or i.t. alone), confirming involvement of opioid receptors. Each of the more receptor subtype-selective antagonists β-FNA (μ), naltrindole (δ), and nor-BNI (κ) attenuated the site/site synergy (Fig. 1). Naltrindole i.t. produced some scratching behaviors that might have masked an even greater antagonism of the synergy. Future work will utilize a different δ-selective antagonist.

It is well known that acetaminophen's analgesic efficacy is related to its plasma concentration and evidence increasingly points to a central site of action (Walker, 1995; Björkman, 1995). Acetaminophen readily passes the blood–brain barrier and distributes throughout the brain and spinal cord (Courade et al., 2001). Our previous finding with naloxone suggested that acetaminophen elicits the activation of one or more endogenous opioid pathways (Raffa et al., 2000). However, acetaminophen does not bind to opioid receptors (Raffa and Codd, 1996) and naloxone does not antagonize acetaminophen's action at a single site (i.c.v. or i.t.); it only attenuates the site/site synergy (Raffa et al., 2000). The present study extends these findings, implicating each of the opioid receptor subtypes and endogenous pathways (endorphin, enkephalin, and dynorphin) to some degree in the overall synergy, possibly in the order $\kappa > \mu \cong \delta$. Future work obtaining full dose–response curves and other techniques will discriminate the relative contribution of each pathway.

These findings support and extend our hypothesis that the analgesic action of acetaminophen includes a component of activation of descending opioid pathways and synergistic interaction at the level of the spinal cord. Validation awaits a demonstration of some selective binding or other action at these sites. The importance extends beyond this single drug, arguably the most widely used analgesic in the world, to elucidation of the third major analgesic pathway in humans.

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References

Björkman, R., 1995. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Acta Anaesthesiol. Scand. 39 (Suppl. 103), 2–44.

Collier, H.O.J., Dinneen, L.C., Johnson, C.A., Schneider, C., 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. 32, 295–310.

Courade, J.-P., Beese, D., Delchambre, C., Hanoun, N., Hamon, M., Eschalier, A., Caussade, F., Cloarec, A., 2001. Acetaminophen distribution in the rat central nervous system. Life Sci. 69, 1455–1464.

Raffa, R.B., Codd, E.E., 1996. Lack of binding of acetaminophen to 5-HT receptor or uptake sites (or eleven other binding/uptake assays). Life Sci. 59, PL37–PL40.

Raffa, R.B., Stone Jr., D.J., Tallarida, R.J., 2000. Discovery of 'self-synergistic' spinal/supraspinal antinoci-ception produced by acetaminophen (paracetamol). J. Pharmacol. Exp. Ther. 295, 291–294.

Raffa, R.B., Stone Jr., D.J., Tallarida, R.J., 2001. Unexpected and pronounced anti-nociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine. Eur. J. Pharmacol. 412, R1–R2.

Walker, J.S., 1995. NSAID: an update on their analgesic effects. Clin. Exp. Pharmacol. Physiol. 22, 855–860.

Warner, T.D., Vojnovic, I., Giuliano, F., Jiménez, R., Bishop-Bailey, D., Mitchell, J.A., 2004. Cyclooxygenases 1, 2, and 3 and the production of prostaglandin I_2: investigating the activities of acetaminophen and cyclooxygenase-2-selective inhibitors in rat tissues. J. Pharmacol. Exp. Ther. 310, 642–647.