Discovery of “Self-Synergistic” Spinal/Supraspinal Antinociception Produced by Acetaminophen (Paracetamol)¹

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ABSTRACT

The mechanism of the analgesic action of one of the world’s most widely used drugs—acetaminophen (paracetamol)—remains largely unknown more than 100 years after its original synthesis. Based on the present findings, this elusiveness appears to have resulted from experimental strategies that concentrated on a single target site or mechanism. Here we report on the use of analyses that we previously developed to investigate possible brain/spinal-cord site-site interaction in acetaminophen-induced antinociception. Spinal (intrathecal) administration of acetaminophen to mice produced dose-related, naloxone-insensitive antinociception with an ED₅₀ value of 137 (S.E. = 23) μg = 907 (S.E. = 153) nmol. In contrast, supraspinal (i.c.v.) acetaminophen administration had no effect. However, combined administration of acetaminophen in fixed ratios to brain and spinal cord produced synergistic antinociception, ED₅₀ = 57 (S.E. = 9) μg, that reverted toward additivity, ED₅₀ = 129 (S.E. = 23) μg, when the opioid antagonist naloxone was given spinaly (3.6 μg = 10 nmol) or s.c. (3.6 mg/kg). These findings demonstrate for the first time that acetaminophen-induced antinociception involves a “self-synergistic” interaction between spinal and supraspinal sites and, furthermore, that the self-synergy involves an endogenous opioid pathway.

An understanding of analgesic mechanisms was significantly advanced during the 1970s when the receptor for morphine was discovered (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973), and the mechanism of aspirin and other nonsteroidal anti-inflammatory drugs was shown to involve inhibition of cyclooxygenase (Smith and Willis, 1971; Vane, 1971). But 25 years later, there is still little understanding regarding the mechanism of the third major analgesic, N-acetyl-p-aminophenol (acetaminophen, paracetamol) (Walker, 1995). Although its analgesic efficacy is known to be related to its plasma concentration (Granados-Soto et al., 1992), little else is known about how this widely used drug works. For example, acetaminophen-induced analgesia is not attributable to an anti-inflammatory action, because a detectable decrease in inflammation occurs only at doses that are much greater than those commonly used. Likewise, it is only a very weak inhibitor of cyclooxygenase in sites when peroxide levels are elevated, such as in inflamed tissue, and does not inhibit neutrophil activation (Hanel and Lands, 1982; Marshall et al., 1987; Abramson and Weissmann, 1989). The recent identification of a second cyclooxygenase isoenzyme (COX-2), which is induced in activated inflammatory cells, has not changed this view, because acetaminophen has minimal effect on COX-2 (prostaglandin H₂ synthase) (Meade et al., 1993; Mitchell et al., 1993).

There is increasing evidence that the major site of analgesic action of acetaminophen might be in the central nervous system. For example, Björkman (1995) lists 22 published or unpublished studies reported since 1971 that support the concept of a central antinociceptive effect of nonsteroidal anti-inflammatory drugs or acetaminophen. Yet when we measured the affinity of acetaminophen for 22 receptor or neurotransmitter neuronal reuptake sites (Raffa and Codd, 1996) at 10 μM concentration in vitro, acetaminophen inhibited less than 10% of specific radioligand binding at any of the sites. Others have reported similar results (Pelissier et al., 1996). These findings limit the possible explanations for acetaminophen’s central analgesic action to mechanisms other than those involving direct binding to the receptor or uptake sites examined. Thus, the actual mechanism of analgesic action remains unknown.

In an effort to investigate why the mechanism of analgesic action of acetaminophen has remained so elusive, we have taken a different approach by using two-site isobolographic analysis, which we have successfully used previously to examine the interaction of two compounds. Specifically, we examined the antinociceptive action of acetaminophen by individually injecting into either spinal [intrathecal (i.t.)] or supraspinal (i.c.v.) sites, in each case using doses that produce concentrations attained in these sites when the drug is

ABBREVIATIONS: i.t., intrathecal; 5-HT₃, 5-hydroxytryptamine₃.
given systemically (Beese et al., 1999) and measuring antinociception. Following this procedure, we made injections into both sites, in various fixed ratio amounts, and again assessed the degree of antinociception. This two-site administration allowed the use of an analysis that we have used in studying the multiplicative action of two separate drugs (Tallarida, 1992; Tallarida and Raffa, 1996; Tallarida et al., 1997; Mc-Cary and Tallarida, 1998). In this case the design of our experiment was aimed at determining whether acetaminophen elicits two-site synergism.

Materials and Methods

Male pathogen-free Swiss-derived albino CrI:CD-1(ICR:BR mice (18–24 g; Charles River Laboratories, Portage, ME) were group-housed (5–10 mice per plastic box) under controlled conditions of temperature, humidity, and 12-h light/dark cycle (lights on 6:00 AM). Food and water were available ad libitum up to the time of the test. Each mouse was used only once and was treated in accordance with the principles expressed in the Declaration of Helsinki. The standard abdominal irritant test described by Collier et al. (1968), with minor modifications, was used. Acetaminophen or vehicle (5% ethanol/distilled water) was injected into the right lateral cerebral ventricle (Haley and McCormick, 1957), into the subarachnoid space by direct puncture of the subverterbral space between L5 and L6 (Hylden and Wilcox, 1980), or both. When both i.c.v. and i.t. injections were made, the i.t. injection was made first, followed immediately by the i.c.v. injection. The volume of injection via either i.c.v. or i.t. route was 5 μl. After 20 min, the mice were injected i.p. with acetylcholine bromide (5.5 mg/kg) and placed into large glass jars and observed for up to 10 min (by an investigator blind to the substance injected centrally) for the occurrence of a single characteristic behavioral response (defined as a wave of constriction and elongation passing caudally along the abdominal wall, accompanied by a twisting of the trunk and followed by extension of the hind limbs) as described by Collier et al. (1968). The absence (inhibition) of this response in 10 min was calculated as percentage of antinociception according to: 100 × (nonresponders/group size). Dose-response curves were generated where possible and the ED50 value and standard error estimates were determined (Litchfield and Wilcoxon, 1949; Tallarida and Murray, 1987). In some cases, naloxone hydrochloride was administered s.c., i.c.v., or i.t. 20 min before acetaminophen or vehicle. When both were administered together centrally, they were injected in the same syringe in total volume of 5 μl.

The distinction between additive and nonadditive action is made from an analysis (Tallarida et al., 1989) that begins with each drug’s single-site dose-effect relation, analyzed from log(dose)-effect curves. If the individual site potencies for the specified effect (e.g., half the maximum antinociceptive effect) are denoted Z1 and Z2, then an additive combination (z1, z2) for producing this effect satisfies the relation,

\[ \frac{z_1}{Z_1} + \frac{z_2}{Z_2} = 1 \]  

(1)

whereas a synergistic combination satisfies

\[ \frac{z_1}{Z_1} + \frac{z_2}{Z_2} < 1. \]  

(2)

When doses are expressed as proportions p1 and p2 of the total amount Zt (both routes), these totals become

\[ Z_t = \frac{1}{p_1 + p_2} \left( \frac{Z_1}{p_1} + \frac{Z_2}{p_2} \right) \]  

(3)

for an additive combination and

\[ \frac{1}{p_1 + p_2} \left( \frac{Z_1}{p_1} + \frac{Z_2}{p_2} \right) < Z_t \]  

(4)

for a superadditive (synergistic) combination. If one of the two sites of administration, say site 1, yields no effect, then the totals are either \[ Z_t = Z_2/p_2 \] for additivity or \[ Z_t < Z_2/p_2 \] for superadditivity. The test that distinguishes additivity from superadditivity involves comparing the calculated additive \[ Z_t = (Z_1/p_1) + (Z_2/p_2) \] to the total dose determined experimentally for a combination with the same proportions. All such determinations are for doses that correspond to 50% of the maximum; thus they are ED50 values. For the application at hand the values of \[ Z_t \] needed in the additive calculation as well as the total dose needed in the actual combination experiment were determined from probit regression applied to percentage antinociception on log dose. Probit regression is a weighted regression procedure that is well established in analyzing quantal dose-effect data. (For detailed discussions, see Finney (1971) and Tallarida (2000).) Graphically, this procedure converts the sigmoid-shaped quantal log dose-effect curve into a straight line from which ED50 and other effect-level doses are readily computed along with the magnitudes of their standard errors.

Results

Acetaminophen was administered i.t. in one set of experiments and administered i.c.v. in a second set of experiments. The i.t. route displayed a clear dose-dependent antinociceptive response, with an ED50 (\[ Z_t \] in the above equations) = 137 (S.E. = 23) μg (Fig. 1). In contrast, i.c.v. acetaminophen produced no effect (protection) at 45 or 90 μg, based on at least 10 animals per dose, and showed only 1 in 10 at the highest dose employed, namely 150 μg (Fig. 1). In the actual dual-site experiment, equal amounts were used, i.e., \[ p_1 = p_2 = 0.5 \], so that the additive total ED50 was calculated as 274 (S.E. = 46) μg. That experiment, based on four doses in this fixed-ratio combination (largest dose pair = 90.7 μg of each), produced the dose-effect relation shown in Fig. 2. It is seen that the experimental combination is laterally displaced to the left and that the degree of displacement, confirmed by a standard probit calculation, was significant (\( P < .05 \)), indicative of synergism between the two sites.

![Fig. 1. Measure of antinociception produced in mice by spinal (i.t.) (filled circles) or supraspinal (i.c.v.) (open circles) administration of acetaminophen. n = 8 to 50 mice per point.](image-url)
The unexpected finding of a multiplicative effect in this dual site dosing regimen suggested a mechanism in which an endogenous component is released. Proceeding on that conjecture we administered the opioid antagonist naloxone by each route and tested its effect on acetaminophen antinociception. Naloxone (3.6 mg, i.c.v.) did not significantly change the combination acetaminophen effect; the ED$_{50}$ ($Z_1$) of the combination was 69 (S.E. = 13) μg. However, naloxone, administered i.t., produced a rightward parallel shift of the dual-site acetaminophen combination's dose-effect curve (Fig. 3), yielding an ED$_{50}$ = 129 (S.E. = 23) μg, a value still indicative of synergism (i.e., less than 274 μg) but not as pronounced as previously shown, ED$_{50}$ of 57 (S.E. = 9) μg, when the blocker was not present. In an additional test we administered a fixed dose of naloxone (3.6 μg, i.t.) along with i.t. doses of acetaminophen. In that experiment the acetaminophen ED$_{50}$ was 133 μg, a value very close to the 137 μg previously found for i.t. acetaminophen alone (graph not shown). These findings prompted an additional experiment in which s.c. naloxone was administered. This regimen also shifted the i.t./i.c.v. acetaminophen dose-response curve to the right (graph not shown). Hence, in the presence of naloxone, whether given i.t or s.c., the site-site synergy reverted toward simple additivity.

As a test of the clinical relevance of these findings, we examined whether s.c. naloxone would antagonize orally administered acetaminophen-induced antinociception. Naloxone (3.6 mg/kg, s.c.) administered 20 min before acetaminophen significantly ($P < .05$) increased the ED$_{50}$ of oral acetaminophen from 121 to 302 mg/kg, consistent with a reduction of the self-synergy (Fig. 4).

**Discussion**

The mechanism of the observed self-synergistic interaction is not known. Clearly, spinal acetaminophen-induced antinociception is not mediated via opioid receptors, because acetaminophen does not bind to opioid receptors (Raffa and Codd, 1996) and we were unable to block i.t. acetaminophen-induced antinociception with spinal administration of the opioid antagonist naloxone. However, some contribution of endogenous opioids at the level of the spinal cord in the self-synergy is implicated, because the self-synergy was significantly attenuated by the administration of naloxone at the spinal level or by s.c. administration, but not by supraspinal administration. Speculation by others about possible central actions of acetaminophen are consistent with these findings. For example, Walker (1995) mentions that a potential mechanism for the central effect of acetaminophen is inhibition of nitric oxide generation, because the antinociceptive effect of acetaminophen is reversed by L-arginine, but not by the inactive isomer D-arginine, and acetaminophen reverses the hyperalgesia induced by either N-methyl-D-aspartate or Substance P (Hunskaar et al., 1985; Björkman, 1995). Recently, Pelissier et al. (1995, 1996) reported that at least a portion of the central antinociceptive effect of acetaminophen in rats might involve spinal cord 5-HT$_3$ (5-hydroxytryptamine$_3$) receptors. Any interaction of acetaminophen with 5-HT would have to be indirect,
because we found that acetaminophen has negligible affinity for 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2, 5-HT2C, 5-HT3, 5-HT4, 5-HT5A or 5-HT7 receptors, or for 5-HT neuronal reuptake sites (Raffa and Codd, 1996). Muth-Selbach et al. (1999) report that acetaminophen decreases spinal PGE2 release. All of these mechanisms are known to "cross-talk" with endogenous opioid systems. The experimental approach that is employed here is an outgrowth of the method of isoboles that has traditionally been used to study combinations of drugs and chemicals that display similar effects. Although this method usually applies to different compounds, it is formally applicable to the use of one compound at two different sites, and this kind of application was the basis for the work of Yeung and Rudy (1980) who employed it in the study of morphine-induced antinociception. The concept is actually quite straightforward. If each of two sites of administration results in a quantifiable effect, the drug's potency for each site can be determined. The concept of additivity is based on the assumption that each site will contribute to the effect in proportion to its individual potency. Thus, if the potency of a substance at one site (say site B) is, for example, 397 units, then the amount at site A needed to get the specified effect level is three times that at site B. Accordingly, a combination (a, b) will act like a + Rb given exclusively at site A, where R is the relative potency (R = A/B = 3 in this example), and the italicized expressions denote those at their named sites. Rearrangement of the equation gives expression of additivity (with notational change) given in eq. 1. When lower doses (a, b), are needed to get the specified effect, one gets the superadditive (synergistic) form expressed by the inequality, eq. 2. This combination interaction, here termed self-synergy, indicates some form of interaction, possibly cross-talk with an endogenous opioid by a mechanism that is not yet clear.

What is clear is that acetaminophen induced spinally-mediated antinociception and displayed antinociceptive self-synergy between spinal and supraspinal sites. These results suggest that acetaminophen-induced analgesia derives in part from a self-synergistic interaction between brain and spinal cord. The virtual lack of acetaminophen antinociceptive activity at the i.c.v. site and the reduced potency at the i.t. site compared with the dual-site effect probably accounts for prior difficulty identifying a mechanism of acetaminophen action. In addition, the results suggest that acetaminophen has two central analgesic mechanisms. The analgesia observed following systemic administration of acetaminophen results from a synergy between released supraspinal components, one of which is opioid-like. The independent contribution of the opioid-like component is small, so that there is minimal effect without acetaminophen at the spinal site. The spinal cord component of acetaminophen-induced antinociception, not an opioid-mediated effect, is activated when acetaminophen enters the spinal site. Synergistic analysis aided the design, quantitation, and illumination of these findings.

References

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