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# Unexpected and pronounced antinociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine

Robert B. Raffa*, Dennis J. Stone Jr., Ronald J. Tallarida

*Temple University School of Pharmacy and School of Medicine, Philadelphia, PA 19140, USA*

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**Abstract**

Acetaminophen was administered to mice by spinal (intrathecal, i.t.) injection alone or with phentolamine (11.3 μg = 0.03 μmol). Acetaminophen produced dose-related antinociception in the abdominal irritant test with an $ED_{50}$ value of 137.2 μg (0.9 μmol). Phentolamine had no effect. For combined administration, the potency of acetaminophen was significantly increased ($ED_{50}$ = 24.4 vs. 137.2 μg), indicative of multiplicative interaction and strong synergism. These results reveal the significant and surprising interaction of spinal cord adrenoceptors or ion channel subtypes with acetaminophen-induced antinociception. © 2001 Published by Elsevier Science B.V.

*Keywords:* Acetaminophen; Antinociception; Synergy

The mechanism of action of the widely prescribed analgesic acetaminophen (*N*-acetyl-*p*-aminophenol; paracetamol) remains largely unknown more than 120 years after the original synthesis of the drug. Unlike traditional or newer non-steroidal anti-inflammatory drugs (NSAIDs), it does not significantly inhibit cyclooxygenase isozymes (either cyclooxygenase-1 or cyclooxygenase-2) at analgesic doses. Acetaminophen crosses the blood–brain barrier and accumulated evidence suggests a central component to its mechanism of action, as summarized by Walker (1995) and Björkman (1995). Despite intriguing potential insights into its central component of action (e.g., Björkman, 1995; Pelissier et al., 1996; and others), the mechanism still remains elusive. At 10 μM, acetaminophen inhibits less than 10% specific radioligand binding at serotonin (5-HT) receptors, at 11 other receptor sites, or at 5-HT and norepinephrine reuptake sites (Raffa and Codd, 1996) and at 100 μM does not inhibit constitutive or inducible nitric oxide (NO) synthase (unpublished data). As part of a larger investigation of acetaminophen's antinociceptive spinal/supraspinal "self-synergy" (Raffa et al., 2000), an unexpected and pronounced synergy was observed between spinal acetaminophen and phentolamine.

Male virus-free Swiss-derived albino Crl: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 06:00 h). Food and water were available ad libitum up to the time of the test. Each animal was used only once and treated in accordance with the recommendations and policies of the National Institutes of Health guidelines regarding the care and use of experimental animals. The abdominal irritant test described by Collier et al. (1968), with minor modifications, was used. The mice were injected with acetaminophen (5% ethanol/distilled water), phentolamine mesylate (11.3 μg), or combinations into the subarachnoid space by direct puncture of the subvertebral space between L5 and L6. The volume of injection was 5 μl. Twenty minutes later, 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 treatment for the characteristic behavioral response (a wave of constriction and elongation passing caudally along the abdominal wall, accompanied by a twisting of the trunk and followed

* Corresponding author. Department of Pharmaceutical Science, Temple University School of Pharmacy, 3307 North Broad Street, Philadelphia, PA 19140, USA. Tel.: +1-215-707-4976; fax: +1-215-707-5228.

*E-mail address:* rraffa@nimbus.temple.edu (R.B. Raffa).

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by extension of the hind limbs). Inhibition of response (%antinociception) was determined according to: $100 \times$ (nonresponders)/(group size). Dose–response data were of the all-or-none (quantal) type and therefore were analyzed with probit analysis (Tallarida, 2000) to yield $ED_{50}$ values and their error estimates. A probability level $P < 0.05$ was the minimum criterion to assess significant differences in all estimated quantities.

Intrathecal (i.t.) acetaminophen produced dose-related antinociception with an $ED_{50} = 137.2 \pm 22.6$ (S.E.M.) μg (Fig. 1). I.t. vehicle or phentolamine had no effect in this test of antinociception. Hence, the expected (additive) $ED_{50}$ of acetaminophen in combination with phentolamine is equal to the $ED_{50}$ of the single agent. Actual tests with a fixed quantity of phentolamine (11.3 μg, i.t.), given with varying quantities of acetaminophen, produced an enhanced dose-related response. This enhancement, indicating pronounced synergism, is evident in the elevated dose–response curve of the analgesic with this co-treatment (Fig. 1). A measure of the synergism is afforded by the reduced $ED_{50}$ of acetaminophen, $24.4 \pm 3.62$, in this case. A more precise statistical assessment follows from the log $ED_{50}$ values in the two cases (2.14 vs. 1.39, from μg quantities) and the calculated 95% confidence limits: (2.02–2.35) for acetaminophen alone and (1.22–1.53) with the phentolamine co-treatment. These results (difference in log $ED_{50}$'s) confirm the synergism that is evident in the graphs. The degree of synergism may be estimated from the relative potency ($R$) calculated at the 50% response level for the two cases: $R = 5.62$ (95% CL = 3.89–7.82), where the confidence limits (CL) were obtained from parallel line analysis of the two probit lines (Tallarida, 2000). In other words, the presence of phentolamine enhanced the potency of i.t. acetaminophen by a factor of approximately 5.6. In the related experiments using either i.t. prazosin, phenylephrine, yohimbine or azepexole concomitantly with acetaminophen, the result in each case was simple additivity (data not shown).

The effect of phentolamine on acetaminophen-induced antinociception was an unexpected finding. We are unaware of previous recognition of a possible spinal interaction between acetaminophen and α-adrenoceptors. The degree of this synergy was also of surprise. Phentolamine produced a 5.6-fold shift in the acetaminophen antinociceptive dose–response curve that did not differ significantly in slope from the acetaminophen regression line. The mechanism responsible for this interaction is not known. The possibility that it involves α-adrenoceptors directly is undermined by the lack of a similar effect by the α-adrenoceptor antagonists prazosin or yohimbine or the agonist azepexole. Perhaps it relates to alternative properties of phentolamine, such as its inhibition of L-type $Ca^{2+}$ channels or ATP-sensitive $K^+$ channels (e.g., Liang et al., 1998), mechanisms which are known to be involved with antinociception.

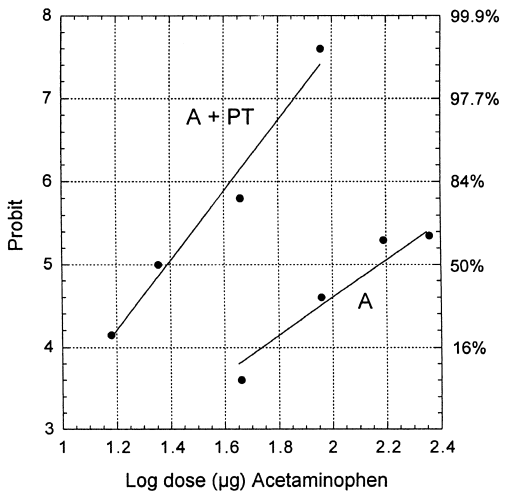


Fig. 1. Probit regression lines for i.t. acetaminophen alone (A) ($y = 2.548x - 0.446$) and for acetaminophen plus 11.3 μg (i.t.) of phentolamine (A + PT) ($y = 3.823x - 0.302$). The right-hand scale shows percentages corresponding to the probit values on the left-hand scale. The leftward shift (elevation) of the regression line was significant, indicating synergism with i.t. co-administration.

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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.

Liang, Y., Wu, B.W., Dun, W., 1998. Blocking effects of phentolamine on L-type calcium current and ATP-sensitive potassium current in guinea pig ventricular myocytes. Acta Pharmacol. Sin. 19, 154–157.

Pelissier, A., Alloui, F., Caussade, C., Dubray, C., Cloarec, A., Lavarenne, J., Eschalier, A., 1996. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine$_3$ receptors: in vivo and in vitro evidence. J. Pharmacol. Exp. Ther. 278, 8–14.

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./Pharmacol. Letts. 59, PL37–PL40.

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

Tallarida, R.J., 2000. Drug Synergism and Dose–Effect Data Analysis. CRC Press, Boca Raton, FL, pp. 91–114.

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