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1,3-di-*o*-Tolylguanidine (DTG) Differentially Affects Acute and Tonic Formalin Pain: Antagonism by Rimcazole

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KEST, B., J. S. MOGIL, W. F. STERNBERG, R. N. PECHNICK AND J. C. LIEBESKIND. *1,3-di-o-Tolylguanidine (DTG) differentially affects acute and tonic formalin pain: Antagonism by rimcazole.* PHARMACOL BIOCHEM BEHAV **52**(1) 175–178, 1995.—The role of the sigma receptor in prolonged pain was examined by assessing the effects of 1,3, di-*o*-tolylguanidine (DTG), a selective sigma receptor ligand, on the formalin test in mice. Formalin injected subcutaneously into the hindpaw produces a biphasic pain response: an acute phase of short duration followed by a longer-lasting tonic phase. DTG (10 mg/kg, IP) potently reduced pain behavior in the acute phase but increased pain behavior in the tonic phase. Rimcazole (5 and 10 mg/kg, IP), a selective sigma receptor antagonist, blocked both the DTG-induced decrease and increase in pain behavior observed in the acute and tonic phases, respectively. These data support previous findings indicating a modulatory role for the sigma receptor in nociceptive processes, and suggest that this receptor differentially modulates acute vs. tonic pain.

Sigma receptor Antinociception Hyperalgesia Mice

WE PREVIOUSLY observed that 1,3-di-*o*-tolylguanidine (DTG) produces modest but significant antinociception on the tail-withdrawal test following systemic administration in mice (18). DTG binds to the sigma receptor with high affinity and is among the most selective ligands known for this site (30). Consistent with a sigma receptor site of action, DTG antinociception in the aforementioned study was blocked in a dose-dependent manner by rimcazole, a selective sigma receptor ligand and putative antagonist (6,7,14,19).

The tail-withdrawal test measures a reflex nociceptive response to noxious heat (11). The nociceptive stimulus in this test is phasic, escapable, and of relatively high intensity. In contrast, tests of tonic pain assess responses to noxious stimuli that are continuous, inescapable, and of moderate intensity. Tonic pain models, because of their longer duration and association with tissue injury, are thought to be more representative of clinical pain states than phasic tests that assess nociceptive threshold changes.

The formalin test of nociception, in which a dilute volume of formaldehyde is injected subcutaneously—typically into the hindpaw of rodents—is one model of tonic pain (13). It evokes a reliable biphasic pain response characterized by paw shaking, licking, or favoring: a brief "acute" phase and a prolonged "tonic" phase. The acute phase begins immediately after formalin injection and lasts 5–10 min. Subsequently, there is a 10-min period of quiescence followed by the tonic phase in which pain behavior can be observed over a period of up to 40 min.

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There is evidence to suggest that phasic and tonic pain are differentially modulated in the central nervous system (CNS) (27). Indeed, antinociceptive efficacy on the tail-withdrawal test does not always predict the effects of drugs on pain of longer duration (1,2,12,13). We therefore evaluated the effects of DTG on both phases of formalin-induced pain. The formalin test is particularly well suited to test drugs like DTG because it is sensitive to drugs with weak analgesic effects on tests employing phasic stimuli (4,15,16,25). The ability of rimcazole to block DTG's effects was also tested.

METHOD

Adult male Swiss-Webster mice, weighing between 30–45 g, were used in all experiments. Animals were housed five to a cage and maintained on a 12L : 12D cycle (lights on at 0800 h) in a temperature-controlled environment, with food and water available ad lib. Each mouse was used only once.

DTG (Aldrich Chemical Co., Milwaukee, WI) was initially dissolved in a small volume of acetic acid, then enough sodium hydroxide and saline were added to neutralize (pH = 7.0) the solution and obtain the desired final concentration. Rimcazole (Research Biochemicals Inc., Natick, MA) was dissolved in 0.9% physiological saline. Animals were randomly assigned to one of six groups (N = 6–9 per group), and received either saline or rimcazole (5 or 10 mg/kg) immediately followed by saline or DTG (10 mg/kg). This dose of DTG has been previously shown to produce modest nociceptive effects on the tail-withdrawal assay with no concomitant motor impairment (18). All drugs were injected IP 15 min prior to behavioral testing, in a volume of 10 ml/kg.

The formalin test was performed as previously described for the rat (13) with some modifications for the mouse (16). Briefly, mice were placed in a clear Plexiglas observation chamber (25 cm diameter; 30 cm height) and allowed to acclimate for 30 min. The plantar surface of one hindpaw was then injected SC with 20 μl of 5% formalin. Animals were continuously monitored for pain responsivity during the acute (0–10 min after formalin injection) and tonic (30–50 min after formalin injection) phases. Pain responsivity was operationally defined as the total amount of time the animal spent licking the injected paw. All testing took place between 1000 and 1400 h.

Mean time spent licking values for each treatment group were calculated for each phase and subjected to analysis of variance (ANOVA). Comparisons between individual group means were made, where appropriate, using the Duncan New Multiple Range post hoc test, $p < 0.05$.

RESULTS

Consistent with our previous findings with higher doses (18), DTG (10 mg/kg) in the present study produced no visible signs of motor dysfunction or malaise alone or in combination with rimcazole. A two-way ANOVA performed on acute phase data demonstrated a significant main effect of DTG, $F(1, 58) = 70.06$, and a significant DTG × rimcazole interaction, $F(3, 58) = 3.45$. Mice receiving saline + DTG displayed significantly less hindpaw licking (a 75% reduction) than mice receiving saline + saline, $F(1, 14) = 65.40$ (Fig. 1A). In addition, the higher rimcazole dose (10 mg/kg) significantly attenuated the effects of DTG on acute phase pain behavior without producing significant effects when administered alone (Fig. 1A). The lower rimcazole dose (5 mg/kg) had no effect on DTG antinociception or acute formalin pain (data not shown).

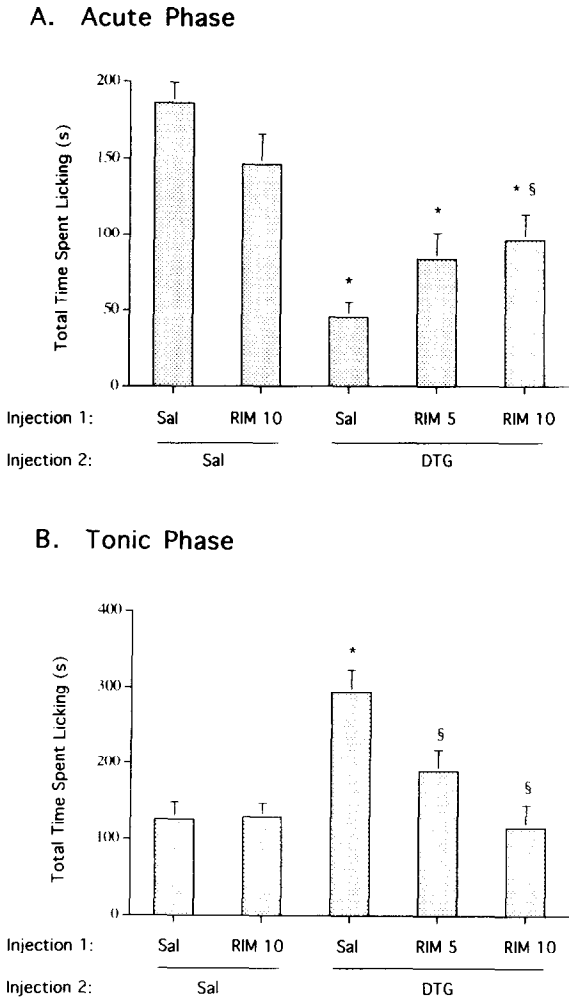

FIG. 1. Effect of DTG (10 mg/kg) on the acute (A: 0–10 min) and tonic (B: 30–50 min) phases of the formalin test following pretreatment with either saline (Sal) or rimcazole [5 mg/kg (RIM 5) or 10 mg/kg (RIM 10)]. Injection 1 immediately preceded injection 2. Bars represent mean total time spent licking the injected hindpaw. *Indicates significant difference from Sal + Sal, $p < 0.05$. §Indicates significant difference from Sal + DTG, $p < 0.05$.

A two-way ANOVA performed on tonic phase data revealed a significant main effect of DTG, $F(1, 45) = 3.36$, and a significant DTG × rimcazole interaction, $F(3, 45) = 9.76$. In contrast to its effects upon acute pain behavior, mice treated with saline + DTG displayed significantly increased licking during the tonic phase (a 133% increase) relative to saline + saline animals, $F(1, 14) = 20.12$ (Fig. 1B). Both doses of rimcazole significantly attenuated the hyperalgesia produced by DTG in the tonic phase in a dose-dependent manner; the 10-mg/kg rimcazole dose completely reversed DTG's hyperalgesic effect. Rimcazole alone had no effect upon tonic phase pain behavior following either the 10-mg/kg (Fig. 1B) or 5-mg/kg (data not shown) dose.

DISCUSSION

Consistent with our previous findings using the tail-withdrawal test (18), DTG produced antinociception on the acute phase of the formalin test following systemic adminis-

tration. The larger magnitude of 10 mg/kg DTG antinociception observed here relative to the tail-withdrawal test may reflect the different physiological processes mediating the two tests (27) and/or the greater sensitivity of the formalin test to drugs with weak analgesic potency against phasically applied noxious stimuli (4,15,16,25). Furthermore, DTG antinociception was antagonized by the prior administration of rimcazole, indicating that DTG antinociception is affected in both nociceptive assays via similar mechanisms. Because both DTG and rimcazole are among the most selective ligands for the sigma receptor (14,30), this site is implicated.

We have previously demonstrated in the mouse and rat that DTG administered systemically at the dose used in the present study produces significant hypothermia (5,18). We do not believe this effect of DTG represents a confound of the observed antinociception. Although the behavioral response to formalin in mice has been previously shown to be sensitive to ambient room temperature (lower temperatures were associated with a decreased pain response), this effect has been observed only during the tonic phase when peripheral inflammatory reactions that contribute to pain develop (24). In the present study, DTG significantly *increased* pain behavior during the tonic phase relative to control animals. Although DTG-induced hypothermia could conceivably contribute to increased tonic phase pain behavior by increasing blood flow to the periphery in an attempt to produce heat loss, we feel that this possibility is unlikely because DTG hyperalgesia was blocked by rimcazole. We have previously demonstrated on the tail-withdrawal test that rimcazole blocks DTG antinociception but not hypothermia in mice (18). Thus, the effects of DTG on pain and temperature regulatory processes are apparently dissociable.

The contrasting effects of DTG on acute and tonic formalin pain may have resulted from the different intervals of time that elapsed between DTG administration and the onset of the acute and subsequent tonic phase (15 and 45 min, respectively). As a result, different concentrations of DTG may have been present in the CNS during the two phases. In a separate experiment, however, DTG given 45 min prior to formalin administration did not produce hyperalgesia during the acute phase (data not shown). Thus, although 45 min had elapsed between DTG administration and the onset of the acute and tonic phases in these two experiments, similar results upon pain behavior were not observed.

Collectively, data from the present study and from our observations using the tail-withdrawal test (18) suggest that DTG-induced increases in pain behavior (i.e., DTG hyperalgesia) are restricted to tonic formalin pain. The differential effects of DTG on acute and tonic formalin pain may result from the distinct neurochemical (26) and neuroanatomical (21,23,29) substrates that have been described for the two phases. The two phases also differ in stimulus quality. Pain behavior during the acute phase is thought to reflect direct chemical stimulation of nociceptors (13,16). The full manifestation of the tonic phase, in contrast, is an expression of local inflammatory processes at the injection site (15,25) in addition to plastic changes (i.e., central sensitization) that occur throughout the neuraxis during the acute phase (9). Presumably, the ability of several classes of drugs (3,8,10,17,20), perhaps including DTG, to produce differential effects on acute and tonic formalin pain is a consequence of distinct substrates underlying acute and tonic pain processes.

Interestingly, Monnet et al. (22) have reported that sigma receptor ligands, including DTG, selectively potentiate *N*-methyl-D-aspartate (NMDA)-induced excitation of hippocampal neurons. 2-APHB, a structural analogue of DTG devoid of affinity for sigma binding sites, was ineffective. Furthermore, only high-affinity sigma receptor ligands blocked DTG potentiation, prompting the authors to conclude that sigma receptor activation modulates NMDA responses in this brain region. NMDA receptors in the hippocampus also contribute to the tonic pain response in the formalin test (21). This conclusion is based on the observation that administration of the NMDA antagonist APV (D-2-amino-5-phosphonopentanoic acid) into the hippocampus attenuates tonic, but not acute, formalin pain behavior. NMDA receptors mediate the plastic changes that occur in the CNS during the acute phase in response to prolonged noxious (formalin) stimulation (9). Thus, NMDA antagonists are only antinociceptive on the tonic phase following systemic (28) or central (10,21) administration. If DTG in the present study acted to increase NMDA receptor excitation in the hippocampus, we would expect the pattern of results reported here; that is, an increase in pain behavior during the tonic (but not acute) phase that is blocked by sigma receptor antagonists such as rimcazole. Given the relative affinities of DTG and rimcazole for the sigma binding site relative to the NMDA receptor, a sigma receptor site of action is indicated for DTG hyperalgesia in the tonic phase of the formalin test. However, a concurrent role for NMDA receptors in DTG hyperalgesia is not inconceivable and warrants further study.

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