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# Sigma sites mediate DTG-evoked hypothermia in rats

S.M. Rawls*, David A. Baron, Ellen B. Geller, Martin W. Adler

*Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140, USA*

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**Abstract**

1,3,-Di-*o*-tolylguanidine (DTG), a sigma agonist, produces hypothermia in rats, but the inability of purported sigma antagonists to block the hypothermia suggests that sites other than sigma may mediate the effect. Recently, *N*-[2-(3,4-dichlorophenyl) ethyl]-*N*-methyl-2-(dimethylamino) ethylamine (BD 1047) has been identified as a functional sigma antagonist in vivo because of its high selectivity for sigma sites and its ability to block DTG-induced dystonia and cocaine-evoked behaviors. Therefore, the present study investigated the effect of BD 1047 on DTG-evoked hypothermia. DTG (1, 10, 20 and 30 mg/kg sc) induced dose-dependent hypothermia. The onset of DTG-induced hypothermia was rapid, with a reduction in body temperature observed 15 min postinjection. To determine whether sigma sites mediated DTG-induced hypothermia, BD 1047 was injected 30 min prior to DTG. BD 1047 (1, 5, 7.5 and 10 mg/kg sc) attenuated the hypothermia in a dose-dependent fashion, thus revealing a sigma site mechanism. The injection of BD 1047 alone did not alter body temperature, suggesting that endogenous sigma systems do not play a tonic role in thermoregulation. The present experiments demonstrate for the first time that a selective sigma antagonist attenuates sigma agonist-induced hypothermia. Moreover, these data provide further evidence that BD 1047 is an effective antagonist for characterizing sigma-mediated effects in vivo.


*Keywords:* Sigma receptors; Sigma sites; Hypothermia; DTG; BD 1047; Body temperature; Thermoregulation; Rats

## 1. Introduction

Sigma receptors were originally proposed based upon the actions of the benzomorphan opiate, (±) SKF 10,047 (Martin et al., 1976). However, (±) SKF 10,047 pharmacology is complex and involves multiple receptor classes. The observation that (±) SKF 10,047 and phencyclidine (PCP) produced similar behavioral responses in several species, and that benzomorphans inhibit the binding of [$^3$H]PCP (Cowan et al., 1979; Brady et al., 1982; Brent, 1991; Holtzman, 1982; Mendelsohn et al., 1984), led to the proposition that sigma ligands exerted their effects by acting at a singular sigma/PCP site (Zukin et al., 1984).

Today, sites defined as sigma are not opioid or PCP (Quirion et al., 1992). Sigma sites have gained heightened acceptance as unique binding sites with a specific pattern of drug selectivity and distinctive distribution throughout the body (Itzhak, 1994; Ryan-Moro et al., 1996). It is now established that two subtypes of sigma sites exist and that these subtypes can be differentiated by their enantioselectivity for benzomorphans. Sigma$_1$ sites are sensitive to (+)-opiates and have high affinity for haloperidol and (+)-pentazocine (Bowen et al., 1990). Sigma$_2$ sites are more sensitive to (−)-opiates than sigma$_1$ sites, but the interaction is naloxone insensitive, distinguishing it from a classical opiate interaction (Hellewell and Bowen, 1990). Endogenous ligands for these sites appear to exist (Su et al., 1986; Patterson et al., 1994), and the existence of a tonically active sigma system has been proposed, especially in relation to analgesia (Chien and Pasternak, 1993; King et al., 1997).

Ligands that interact with sigma sites have been reported to produce hypothermia (Kest et al., 1995; Bejanian et al., 1990, 1991; Hjorth et al., 1985; Clark et al., 1981). Still, attempts to define a functional role for sigma sites in thermoregulation have been hampered by a lack

* Corresponding author. Tel.: +1-215-707-5305; fax: +1-215-707-1904.

*E-mail address*: smrawls28@hotmail.com (S.M. Rawls).

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of selective ligands. The use of 1,3,-di-*o*-tolylguanidine (DTG) has provided further insight. DTG displays a high affinity for sigma receptors that is several hundredfold greater than for PCP and NMDA receptors (Weber et al., 1986; Contreras et al., 1988; Steinfels et al., 1989). DTG produces behaviors that are dissimilar to PCP-evoked behaviors and physiologic effects that are inconsistent with a PCP or NMDA receptor locus (Brent, 1991; Herling et al., 1981; Holtzman, 1980, 1989). Finally, the systemic injection of DTG induces hypothermia in rats at doses that do not produce gross alterations in behavior (Bejanian et al., 1991; Woods et al., 1987).

Previous studies with antagonists have failed to attribute a sigma site mechanism to DTG-evoked hypothermia. Rimcazole, which prevents DTG-evoked effects in vivo (Ferris et al., 1986; Kest et al., 1995; Coccini et al., 1991) and is supposed to have sigma antagonist activity (Ferris et al., 1986), did not alter DTG-induced hypothermia in rodents (Bejanian et al., 1991; Kest et al., 1995). Another reported sigma antagonist, BMY 14802 (Ferris et al., 1986), potentiated DTG-induced hypothermia in rats (Bejanian et al., 1991). Moreover, the injection of BMY 14802 by itself decreased body temperature in rats (Bejanian et al., 1991) and mice (Bristow et al., 1991). It is unclear as to why DTG-evoked hypothermia was insensitive to rimcazole and BMY 14802. One possibility is that rimcazole has low affinity for the subtype of sigma site that mediated DTG-evoked hypothermia. Moreover, rimcazole and BMY 14802 have been reported to interact with receptor systems, other than sigma, that affect thermoregulation (Bristow et al., 1991; Steinfels et al., 1989; Matthews et al., 1986; Eaton et al., 1996; Hadfield and Milio, 1987; Mallick and Alam, 1992; Husbands et al., 1997; Nemmani et al., 2001; Malone and Taylor, 2001).

*N*-[2-(3,4-dichlorophenyl) ethyl]-*N*-methyl-2-(dimethylamino) ethylamine (BD 1047) has been identified as a functional sigma antagonist because of its ability to block the motor effects of cocaine (McCracken et al., 1999; Romieu et al., 2000). In addition, BD 1047 blocked DTG-evoked dystonia, vacuous chewing and facial tremors (Matsumoto et al., 1995, 1999; Tran et al., 1998). Unlike rimcazole and BMY 14802, BD 1047 has not been reported to produce in vivo effects by interacting with other neurotransmitter systems. This observation is supported by the fact that BD 1047 has a high affinity for $sigma_1$ and $sigma_2$ sites (Matsumoto et al., 1995) and is several hundredfold more selective for sigma binding sites versus opioid, PCP, muscarinic, dopamine or serotonin sites (McCracken et al., 1999; Matsumoto et al., 1995). Thus, we chose to use BD 1047 to elucidate the role, if any, that sigma sites play in DTG-induced hypothermia.

The present study investigated the effect of BD 1047 on DTG-induced hypothermia. In addition, we investigated the effect of BD 1047 on body temperature to determine whether sigma sites tonically alter body temperature.

## 2. Materials and methods

### 2.1. Animals

All procedures on animals were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (Zivic-Miller, Pittsburgh, PA, USA) weighing 250–350 g were used in the present study. Rats were housed three per cage for a minimum of 5 days before experimental use. They were fed rat chow and water ad libitum and were kept on a 12-h light/dark cycle. The animal room was maintained at 22±2 °C and 50% relative humidity.

### 2.2. Drugs

DTG was obtained from Sigma-Aldrich (St. Louis, MO, USA). BD 1047 was purchased from Tocris Cookson (Ballwin, MO, USA). DTG and BD 1047 were dissolved in a pyrogen-free, 3% lactic acid/saline vehicle.

### 2.3. Experimental protocol

Body temperature experiments were initiated between 9 and 10 a.m. Rats were placed in an environmental room that was maintained at a constant temperature of 21±0.3 °C and relative humidity of 52±2%. Following a 1-h acclimation interval, baseline temperature measurements were taken (Xin et al., 1997). A thermistor probe was lubricated and inserted approximately 7 cm into the rectum. A digital thermometer was used to record body temperature. Rats were unrestrained during the temperature readings, with only the tail being held gently between two fingers. Body temperature was taken every 30 min for 60 min. The first body temperature measurement was discarded to allow for adaptation to the technique. The next two measurements were averaged to establish a baseline body temperature. Next, DTG (1, 10, 20 or 30 mg/kg) or vehicle was injected subcutaneously. These doses of DTG have been reported to produce significant hypothermia in rats (Bejanian et al., 1991). Following the injection of DTG, body temperature was measured for 240 min at intervals of 15, 30, 45, 90, 120, 180 and 240 min postinjection.

In separate experiments, rats were pretreated with BD 1047 to determine whether the DTG-induced hypothermia was mediated by sigma receptors. Although the effects of the systemic injection of BD 1047 have not been reported in rats, BD 1047 (1–40 mg/kg ip) prevented cocaine-induced convulsions and conditioned place preference in mice (McCracken et al., 1999; Romieu et al., 2000). Since 5 mg/kg blocked cocaine-evoked convulsions as effectively as 30 mg/kg, we chose a dose range of 1–10 mg/kg BD 1047. Following a 60-min baseline interval, BD 1047 (1, 5, 7.5 or 10 mg/kg sc) or vehicle was injected.

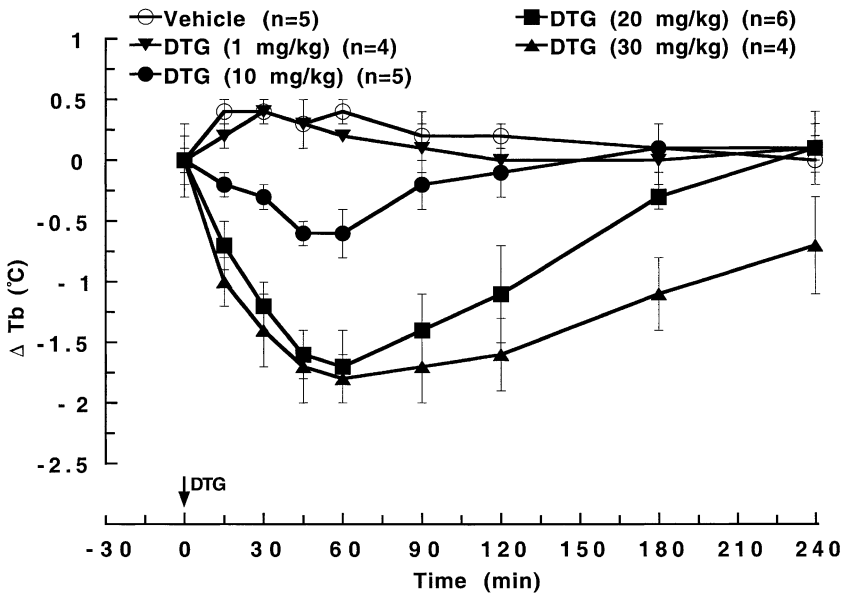


Fig. 1. DTG (1–30 mg/kg sc) produces hypothermia in a dose-dependent manner. DTG was injected at 0 min, as indicated by arrow. Data are expressed as means±S.E. of body temperature. *n*: Number of rats; $\Delta T_b$: change in body temperature from baseline (Time 0). Groups receiving 20 and 30 mg/kg DTG displayed significant hypothermia relative to the saline group, $P<.05$.

Thirty minutes later, DTG (20 mg/kg sc) was injected. Body temperature was measured 15, 30, 45, 60, 90, 120 and 180 min postinjection.

A third set of experiments investigated the effect of BD 1047 alone on body temperature. BD 1047 (1, 5, 7.5 or 10 mg/kg sc) was injected after a 60-min baseline interval. Body temperature was measured for 180 min.

### 2.4. Data analysis

Two consecutive body temperature readings were recorded and averaged to establish a baseline temperature prior to the administration of drugs. Data were presented as means±S.E. of body temperature. Statistical analysis of differences between groups was determined by a one-way

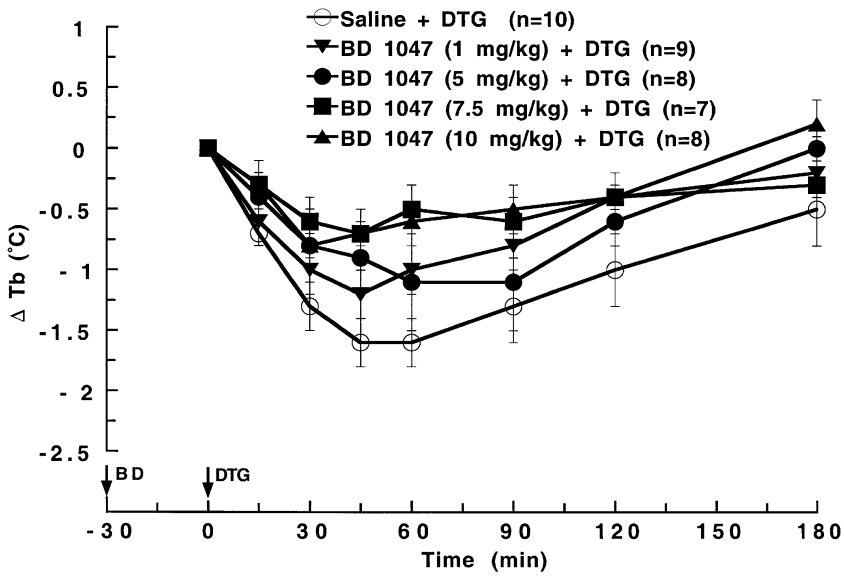


Fig. 2. BD 1047 (1–10 mg/kg sc) attenuates DTG (20 mg/kg sc)-induced hypothermia. DTG was injected at 0 min. BD 1047 was injected 30 min prior to DTG. Data are expressed as means±S.E. of body temperature. *n*: Number of rats; $\Delta T_b$: change in body temperature from baseline (Time 0). Groups receiving pretreatment with 7.5 or 10 mg/kg BD 1047 displayed significantly less hypothermia relative to the saline+DTG group, $P<.05$.

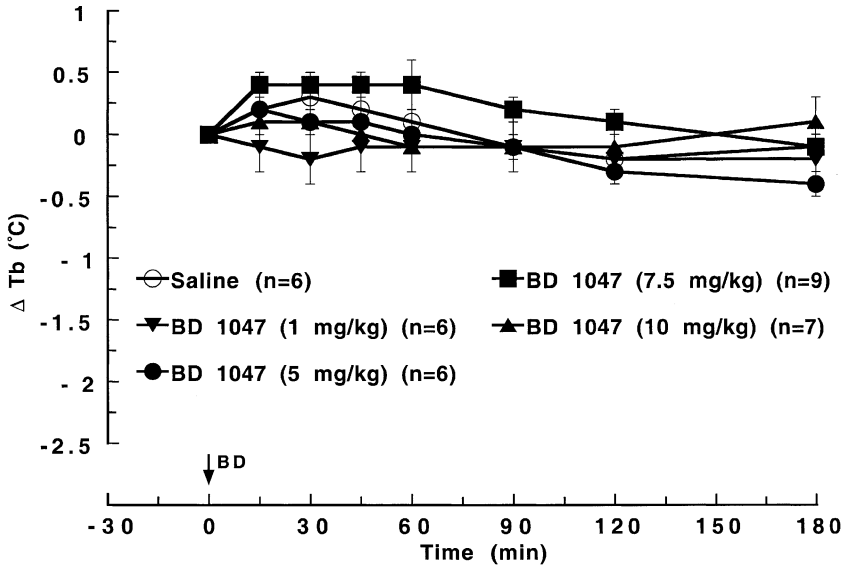


Fig. 3. BD 1047 (1–10 mg/kg sc) by itself does not alter body temperature. BD 1047 was injected at 0 min, as indicated by arrow. Data are expressed as means±S.E. of body temperature. *n*: Number of rats; $\Delta T_b$: change in body temperature from baseline (Time 0). Groups receiving BD 1047 did not significantly affect body temperature relative to the group receiving saline, $P<.05$.

analysis of variance (ANOVA) with repeated measures followed by a Dunnett's post hoc test. A value of $P<.05$ was considered to be statistically significant.

## 3. Results

The subcutaneous injection of DTG at doses of 1–30 mg/kg produced hypothermia in a dose-dependent manner [$F(4,40)=24.13$, $P<.0001$] (Fig. 1). Post hoc analysis demonstrated that doses of 20 and 30 mg/kg produced significant hypothermia relative to saline ($P<.05$). The lower doses, 1 and 10 mg/kg, did not significantly affect body temperature relative to saline. The onset of DTG-induced hypothermia was rapid, with a reduction in body temperature beginning 15 min postinjection. A dose of 20 mg/kg DTG produced a peak hypothermia of 1.7±0.3 °C 60 min postinjection. Similarly, 30 mg/kg DTG produced a maximal hypothermia of 1.8±0.2 °C 60 min postinjection. Thereafter, body temperature recovered gradually and returned to predrug levels 180–240 min after the injection of 20 mg/kg DTG. The hypothermia produced by 30 mg/kg DTG was more persistent than that produced by 20 mg/kg, and body temperature did not return to baseline during the measurement interval. Following the injection of DTG, rats remained quiet and inactive for the duration of the measurement interval. Moreover, we did not observe any visible behavioral effects, such as ataxia, stereotypy, circling or dystonia, following DTG administration.

To determine whether sigma sites played a role in the hypothermic effects of DTG, BD 1047 (1–10 mg/kg) was injected 30 min prior to DTG (Fig. 2). BD 1047 significantly attenuated DTG-evoked hypothermia in a dose-sensitive manner [$F(4,35)=14.77$, $P<.0001$]. Post hoc analysis revealed that only the highest doses, 7.5 and 10 mg/kg, of BD 1047 significantly attenuated the hypothermic effects of DTG ($P<.05$) (Fig. 2). DTG-induced hypothermia was not significantly affected by pretreatment with lower doses, 1 and 5 mg/kg, of BD 1047. These data suggest that DTG produces hypothermia by activating sigma sites.

The effect of BD 1047 (1–10 mg/kg) alone on rectal temperature was determined (Fig. 3). A one-way ANOVA with repeated measures revealed significant differences among the means [$F(4,35)=4.69$, $P=.005$] (Fig. 3). Subsequent post hoc analysis with Dunnett's test, however, revealed that none of the doses of BD 1047 significantly altered body temperature relative to saline (Fig. 3). Moreover, BD 1047 elicited no observable behavioral effects. Our data do not support the existence of an endogenous sigma system that tonically regulates body temperature.

## 4. Discussion

The experiments reported here demonstrate that the sigma site agonist, DTG, produces hypothermia in a dose-dependent manner. The selective sigma site antagonist, BD 1047, attenuated the DTG-induced hypothermia, thus revealing a sigma site mechanism. The injection of BD 1047 by itself did not alter body temperature, indicating that the endogenous sigma system does not tonically regulate body temperature.

### *4.1. Subcutaneous injection of DTG produces dose-dependent hypothermia*

Consistent with previous studies, the subcutaneous injection of DTG caused a decline in body temperature

(Bejanian et al., 1991; Kest et al., 1995). The hypothermia was dose dependent, and the onset of hypothermia was rapid, with a marked reduction in body temperature occurring 15 min postinjection. DTG produced peak hypothermia within 45–60 min postinjection, regardless of the dose. The duration of DTG-evoked hypothermia was persistent, with body temperature gradually approaching predrug values by 3–4 h postinjection.

Rats remained quiet and inactive following the injection of DTG, and we did not observe gross alterations in behavior. Previous studies concur with our observations (Bejanian et al., 1991; Woods et al., 1987), although anxiogenic and discriminative stimulus effects have been reported (Lai et al., 1989; Holtzman, 1989). In addition, high doses of DTG (30 mg/kg), in combination with kappa opioid agonists, have been reported to induce oral activity, such as vacuous chewing and teeth grinding (Brent, 1993). When DTG is administered intracerebrally, rather than systemically, rats display ataxia (Contreras et al., 1988; Bejanian et al., 1991) and hypothermia (Bejanian et al., 1991). Thus, the effects of DTG on gross behavior may be dependent on the route of administration and dose. It is possible that the biodistribution of DTG, such as the ability to cross the blood–brain barrier, underlies the discrepancy in hypothermic, behavioral and motor effects. This supposition, however, is not supported by data indicating that systemically administered DTG displaces 3-(+)-PPP from rat brain (Koe et al., 1989). Nonetheless, the present data raise the possibility that distinct mechanisms may mediate DTG-induced alterations in body temperature and gross behavior.

### 4.2. BD 1047 attenuates DTG-induced hypothermia

BD 1047 attenuated DTG-induced hypothermia, thus revealing a sigma mechanism. This is the first demonstration that a sigma antagonist attenuates the hypothermia produced by a sigma agonist. These results also confirm that BD 1047 is an effective antagonist for characterizing sigma-mediated effects in vivo (Romieu et al., 2000; McCracken et al., 1999; Matsumoto et al., 1995; Tran et al., 1998). Pharmacological data are supported by binding studies, which have demonstrated that BD 1047 has a high affinity for both subtypes of sigma sites and is several hundredfold more selective for these sites versus opioid, PCP, muscarinic, dopamine or serotonin receptors (Matsumoto et al., 1995; McCracken et al., 1999).

The present data are somewhat at odds with previous studies, which have reported that rimcazole does not reduce the hypothermic effects of DTG (Bejanian et al., 1991; Kest et al., 1995). Bejanian et al. (1991) suggested that rimcazole lacked sigma antagonist activity at the doses (25 mg/kg) used or that DTG produced hypothermia through a sigma-independent mechanism. Indeed, rimcazole has an affinity for sigma sites lower than that of BD 1047, as well as lower selectivity (Largent et al., 1988; Tam et al., 1992). Rimcazole may also affect body temperature through neurotransmitter systems other than sigma (Eaton et al., 1996; Hadfield and Milio, 1987; Mallick and Alam, 1992; Nemmani et al., 2001; Chipkin, 1988; Malone and Taylor, 2001; Nava et al., 2000). Thus, rimcazole may act at nonsigma sites to produce effects that could interfere with its ability to alter DTG-evoked hypothermia.

Another purported sigma antagonist, BMY 14802, potentiated the hypothermic effect of DTG (Bejanian et al., 1991). In addition, the administration of BMY 14802 by itself evoked hypothermia (Bejanian et al., 1991; Bristow et al., 1991). Bristow et al. (1991) suggested that serotonin, not sigma, sites mediate the behavioral profile of BMY 14802 (Bristow et al., 1991). This idea is supported by binding data, which indicate that BMY 14802 has similar affinities for 5-$HT1_A$ receptors and sigma sites (Bristow et al., 1991). BMY 14802 has also been reported to interact with dopaminergic systems (Steinfels et al., 1989; Matthews et al., 1986). Those data and the well-established role of serotonin and dopamine in thermoregulation (Malone and Taylor, 2001; Nava et al., 2000) suggest that BMY 14802 may have produced hypothermia by a nonsigma mechanism.

The much higher affinity of BD 1047 versus rimcazole for sigma receptors probably explains the differences between the present data and previous results. Still, variations in the experimental design must also be considered. One discrepancy is the time interval between the injection of antagonist and DTG. We injected BD 1047 30 min prior to DTG, while Kest et al. (1995) coadministered rimcazole and DTG to mice. Similarly, Bejanian et al. (1991) administered DTG to rats immediately after the injection of rimcazole (Bejanian et al., 1991). Thus, BD 1047 may have blocked sigma sites more effectively than rimcazole because of the 30 min pretreatment. Still, it is a matter of speculation as to whether the variations in pretreatment times contributed to these divergent results.

While it is beyond the scope of the present investigation to elucidate the locus of the sigma-mediated hypothermia, several lines of evidence implicate the hypothalamus, which is thought to play a major role in thermoregulation (Boulant, 1981). Neurons expressing sigma site mRNA are distributed in hypothalamic regions where autoradiographic binding studies have demonstrated dense sigma site expression (Mei and Pasternak, 2001; Largent et al., 1986; Bouchard and Quirion, 1997). $Sigma_1$ binding sites were particularly abundant in the hypothalamic nuclei (Bouchard and Quirion, 1997). These data suggest that sigma sites and thermosensitive neurons in the hypothalamus are closely associated and support a role for sigma sites in the mediation of hypothermia (Boulant, 1981). Still, lack of information concerning the cellular localization of sigma sites in the hypothalamus and other regions of the CNS precludes determining the anatomical locus of the sigma-mediated hypothermia.

It is worth mentioning that BD 1047 did not block completely the hypothermic response to DTG. This sug-

gests that a component of DTG-evoked hypothermia is resistant to pretreatment with BD 1047. Indeed, DTG may have activity at sites other than sigma, and interactions at those sites may contribute to the hypothermic effects of DTG. For example, DTG can antagonize carbachol-induced contractions in the guinea pig ileum, demonstrating that it has antagonist activity at cholinergic muscarinic receptors (Vargas and Pechnick, 1991). Along similar lines, DTG can displace the binding of the muscarinic ligand [$^3$H]-methylscopolamine in guinea pig cortical membranes ($K_i$=2 μM), and it is possible that such concentrations could be achieved by doses of 20–30 mg/kg (Bejanian et al., 1991; Vargas and Pechnick, 1991). Moreover, in vivo microdialysis studies have demonstrated that systemically administered DTG elevates extracellular acetylcholine and dopamine levels in the rat frontal cortex and striatum, respectively (Matsuno et al., 1993; Patrick et al., 1993). Thus, nonsigma sites may have played a role in the hypothermic effects of DTG.

### *4.3. BD 1047 does not alter body temperature*

The injection of BD 1047 by itself did not alter body temperature in the present study. These data indicate that sigma sites do not tonically regulate body temperature. The lack of effect of BD 1047 on body temperature suggests that BD 1047 is devoid of sigma agonist activity at the doses used in the present study. This lack of sigma agonist activity by BD 1047 suggests that the recognition and activation of sigma sites are separable events. Previous studies have characterized BD 1047 as a novel sigma antagonist because of the following: (1) high affinity for $sigma_1$ and $sigma_2$ sites; (2) several hundredfold higher selectivity for sigma sites than at least nine other CNS receptors; (3) blockade of in vivo effects attributable to DTG; and (4) negligible neurotoxic effects in vivo (Matsumoto et al., 1995, 1999; McCracken et al., 1999; Tran et al., 1998; Zambon et al., 1997).

It should be pointed out that BD 1047 does exhibit high affinity for the β-adrenergic receptor, and β-adrenergic antagonists have been reported to produce hypothermia (Banet et al., 1978; Ben-Uriah et al., 1981). The fact that BD 1047 did not alter body temperature, however, does not support a role for the β-adrenergic system in the effects of BD 1047 on thermoregulation.

## 5. Conclusion

The present experiments indicate that BD 1047, a sigma antagonist, attenuates the hypothermic effect of DTG, thus implicating sigma sites as the locus of the hypothermia. The present data is the first evidence that sigma agonist-induced hypothermia is suppressed by the blockade of sigma sites. These data, taken together with several recent reports, suggest that BD 1047 is a new and highly effective antagonist that may provide useful insight into the functional role of sigma sites in hypothermia and other physiological effects.

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