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Pronounced hypothermic synergy between systemic baclofen and NOS inhibitor

Scott M. Rawls*, David A. Baron, Teresa Gomez, Kyle Jacobs, Ronald J. Tallarida

Department of Pharmaceutical Sciences and Pharmacology, Temple University School of Pharmacy and Medicine, 3307 North Broad Street, Philadelphia, PA 19140, United States

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Abstract

Baclofen was administered to rats systemically (intraperitoneal, i.p.) by itself or with L-NAME. Baclofen (1–7.5 mg/kg, i.p.) evoked dose-dependent hypothermia. L-NAME (50 mg/kg, i.p.) was ineffective. For combined administration, L-NAME increased the relative potency of baclofen (F=10.77, p<0.05), indicating multiplicative interaction and synergism. The present data reveal a surprising and significant interaction between nitric oxide synthase (NOS) and baclofen-induced hypothermia.

Keywords: Baclofen; L-NAME; Synergy

Baclofen activates $GABA_B$ receptors to induce a myriad of biological actions, including antinociception, motor impairment, muscle relaxation, and hypothermia. With respect to body temperature, the systemic administration of baclofen induces hypothermia in rats and humans that is mediated in part by $GABA_B$ receptors (Zarrindast and Oveissi, 1988; Perry et al., 1998; Rawls et al., 2004). The mechanism is not entirely clear; however, since a lack of effect on body temperature and hyperthermia have been reported following baclofen administration to rats (Sancibrian et al., 1991; Zarrindast and Oveissi, 1988).

The complex thermoregulatory effects of baclofen suggest that endogenous systems other than GABA play a role. One candidate is nitric oxide (NO), a critical second messenger that plays a role in thermoregulation, hypertension, respiration, and inflammation. NO is synthesized by the enzyme nitric oxide synthase (NOS) and is involved in hyperthermia and hypothermia (Benamar et al., 2001; Steiner et al., 1998). NO and GABA systems interact in vivo, with perhaps the most convincing proof being the demonstration that a NO component antagonizes baclofen-evoked impairment of learning and memory in rats (Pitsikas et al., 2003). As part of a larger investigation into in vivo GABA–NO interactions, a pronounced synergy was observed between baclofen and L-NAME, a NOS inhibitor.

Male Sprague–Dawley rats (200–250 g) were housed one per cage for 5 days before experimental use under controlled conditions and a 12-h light/dark cycle. Each rat was used once and treated in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Between 9 and 10 AM on the morning of the experiment, rats were placed in an environmental room 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. A thermistor probe was lubricated and inserted 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 between two fingers. Body temperature was recorded every 30 min during a 90-min baseline interval. Rats were injected with saline, baclofen (1–7.5 mg/kg, i.p.), L-NAME (50 mg/kg, i.p.) or a combination of baclofen (1–7.5 mg/kg) and L-NAME (50 mg/kg). Body temperature was recorded every 15 for 60

* Corresponding author. Tel.: +1 215 707 4942; fax: +1 215 707 3678.
E-mail address: scott.rawls@temple.edu (S.M. Rawls).

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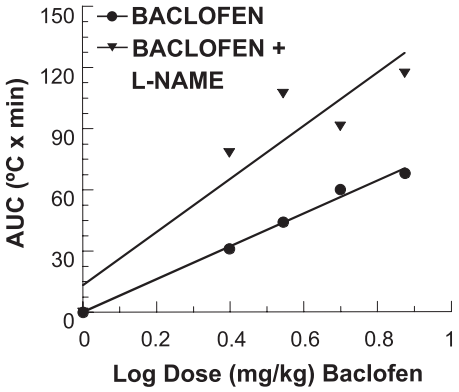

Fig. 1. Regression analysis of baclofen (1, 2.5, 3.5, 5, and 7.5 mg/kg, i.p.) alone (●) (y=80.026x+0.203) and in combination with a non-hypothermic dose of L-NAME (50 mg/kg, i.p.) (▼) (y=129.73x+13.170). The effect is AUC from 0 to 60 min and is determined from at least eight animals. The combination dose–effect curve for baclofen plus L-NAME is significantly elevated (F=10.8, p<0.05) above the curve for baclofen alone, thus indicating synergism for the drug interaction.

min post-injection. Three consecutive body temperature readings were averaged to establish a baseline temperature before injection, and the change from baseline was determined. Area under the body temperature time curve ($AUC_{0–60}$) from 0 to 60 min post-administration was determined using the trapezoidal rule. A probability level of p<0.05 was considered to be statistically significant.

Intraperitoneal (i.p.) injection of baclofen (1–7.5 mg/kg) produced dose-related hypothermia. I.p. injection of vehicle or L-NAME (50 mg/kg) was ineffective. Hence, the expected (additive) hypothermic effect of baclofen in combination with L-NAME is equal to the hypothermia produced by baclofen alone. Therefore, the analysis becomes one in which the dose–effect curve of the active agent is statistically compared before and after the addition of the inactive agent. Actual experiments with a fixed dose of L-NAME (50 mg/kg) given with five doses of baclofen (1–7.5 mg/kg) produced an enhanced dose-related effect. The effect is $AUC_{0–60}$. Regression lines (effect on log dose) for baclofen alone and in combination with L-NAME are shown in Fig. 1. The significant leftward shift in the combination curve means that an interaction has occurred (Tallarida, 2001). A simply additive interaction would cause the same dose–response relation. Statistical comparison by ANOVA revealed a significant difference (F=10.77, p<0.05) between the baclofen and combination regression curves, indicating synergism for the interaction.

To our knowledge, the present data are the first to demonstrate synergy between baclofen and a NOS inhibitor (L-NAME). In related experiments, another NOS inhibitor, 7-nitroindazole, enhanced baclofen-induced hypothermia, providing further support for NOS and baclofen interactions (data not shown). The mechanism for the interaction is unknown, but it is possible that a decrease in NO synthesis enhances the hypothermic response to baclofen. When considered in a context beyond thermoregulation, these results support the existence of GABA–NOS interactions and suggest a role for NOS in baclofen pharmacology.

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