



EFFECTS OF GABA_A-ACTIVE AGENTS ON THERMOREGULATION IN RATS

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ABSTRACT

PURPOSE: Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter, which is widely distributed throughout the mammalian brain including hypothalamus. Immunohistochemical research have reported GABA-ergic neurons and GABA_A-receptors on the neurons of the preoptic area of anterior hypothalamus (PO/AH). The aim of this study was to investigate the influence of GABA_A-ergic substances on thermoregulation in rats. **METHODS:** We have studied the effects of GABA_A-active agents, muscimol and diazepam on core body temperature in rats after systemic administration (intraperitoneally, i.p.). Body temperature was measured with thermistor probes (TX8) and monitored on multichannel recorder THERMEX 16. **RESULTS:** Intraperitoneal injection of muscimol or diazepam has produced dose-dependent hypothermia. Hypothermic effect of muscimol was inhibited by pretreatment of bicuculline, a competitive antagonist of GABA_A-receptors. Diazepam induced hypothermia was antagonized by pretreatment of animals with flumazenil, a competitive antagonist of benzodiazepine receptors. **CONCLUSION:** Hypothermia induced by muscimol or diazepam suggest involvement of GABA_A receptors in the processes of thermoregulation.

Key words: GABA_A-active drugs, muscimol, diazepam, thermoregulation, rats.

INTRODUCTION

Thermoregulation is the complex physiologic process involving both central and peripheral autonomic mechanisms. The primary thermoregulatory center resides in the preoptic area of the hypothalamus and controls the balance between heat gain and heat loss.

Many experimental studies suggest the participation of GABA in the processes of thermoregulation. Immunohistochemical research have reported GABA-ergic terminals and GABA_A-receptors on the neurons of the preoptic area of the anterior hypothalamus (PO/AH) (1-2). Systemic or central administration of either GABA or GABA_A and GABA_B agonists usually produce hypothermia, whereas antagonists of GABA_A and GABA_B receptors induce hyperthermia (3-6).

Muscimol is produced naturally in the

mushrooms [Amanita muscaria](#), [Amanita pantherina](#), and [Amanita gemmata](#), along with [muscarine](#), [muscazone](#), and [ibotenic acid](#) (7). Muscimol is a potent [GABA_A agonist](#), activating the [receptor](#) for the brain's major inhibitory [neurotransmitter GABA](#). Muscimol binds to the same binding site on the GABA_A receptor complex as GABA itself, as opposed to other GABAergic drugs such as [barbiturates](#) and [benzodiazepines](#) which bind to separate regulatory sites (8).

Diazepam, a benzodiazepine derivative is mainly used to treat anxiety, insomnia, seizures including status epilepticus, and symptoms of acute alcohol withdrawal. It is also used as a premedication for inducing sedation, anxiolysis or amnesia before certain medical procedures (e.g., endoscopy). Diazepam binds at benzodiazepine binding site that is located on the postsynaptic [GABA_A receptors](#) and enhances binding of GABA. Diazepam increases the frequency of GABA-mediated chloride ion channel opening (9).

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The purpose of the present study was to assess the effects of GABA_A-active agents, muscimol, a selective GABA_A-agonist, and diazepam, a positive allosteric modulator of GABA_A-ergic receptors, on thermoregulation in rats.

MATERIALS AND METHODS

Substances. The following substances were used: Muscimol (Sigma), Diazepam (Sigma), R(-)-Bicuculline methiodide (Sigma), and Flumazenil (Sigma). In the present study all agents were administered intraperitoneally (i.p.) in a volume of 0.2 ml/100 g body weight. Bicuculline was injected 10 min before GABA_A-agonists (muscimol or diazepam) administrations. Flumazenil was injected immediately before application of diazepam. The rats from control group were treated with 0.9% sodium chloride (NaCl) in a volume of 0.2 ml/100 g body weight.

Experimental animals. The experiments were carried out on male Wistar rats (weight range 200-220 g), which were divided into groups of 6-8 rats each. Rats were maintained on a standard 12 h light/dark cycle and allowed food and water ad libitum. Individual rats were used in one experiment. The experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals.

Body temperature measurements. All body temperature experiments started at 10 a.m. and were conducted at ambient temperature of 22 ± 1°C. Body temperature was measured with

thermistor probes (TX8) and monitored on multichannel recorder Iso-Thermex 16 (Columbus Instruments, USA). The thermistor probes were lubricated and inserted rectally to a depth of 6 cm. Before drug administration the initial temperature of the animals was determined. Body temperatures were recorded at 30, 60, 90, 120, 150 and 180 min following injection of the tested substances. The movements of the rats were slightly restricted, as previously described (10).

Data analysis. The results were calculated as delta (Δ) values (mean Δ values ± S.E.M.). Transformed data were analyzed with two-way analysis of variance (ANOVA). For statistical significance a Student's t-test was used. In all cases, values of P<0.05 were considered to be statistically significant.

RESULTS

Effects of muscimol on core body temperature in rats after systemic application

Intraperitoneal injection of muscimol (2 and 4 mg/kg) caused dose-dependent decrease in the core body temperature of the rats (Fig. 1). The hypothermic effect was started soon after injection with a maximum observed at 90 min (P < 0.01) and attenuation until the 150 min. The control group treated with saline showed no change in body temperature of the rats. Pretreatment with bicuculline (2 mg/kg i.p.), a selective GABA_A antagonist inhibit muscimol-induced hypothermia in rats (Fig. 1).

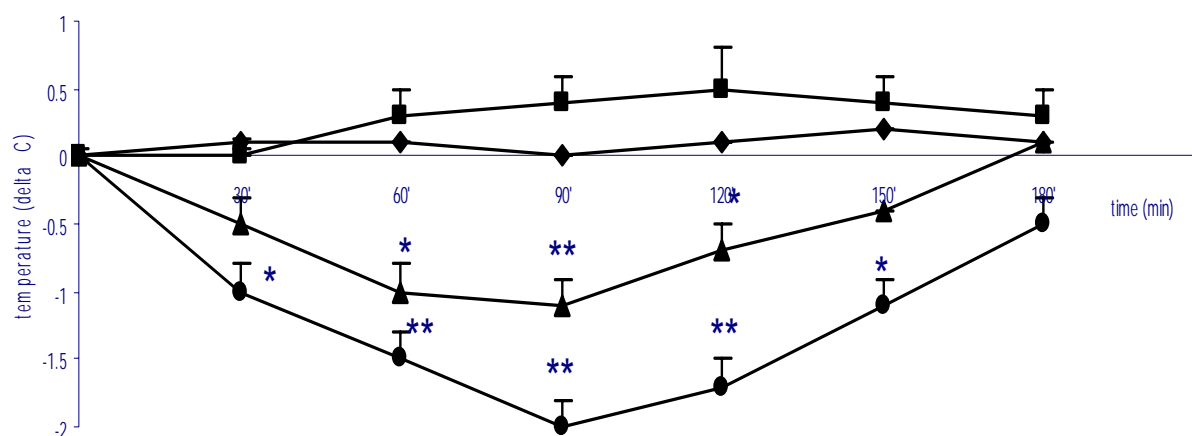


Fig. 1. Effects of intraperitoneal application (i.p.) of muscimol and bicuculline on body temperature in rats. Mean change (temperature delta °C) after i.p. administration of: ▲ Muscimol 2 mg/kg, ● Muscimol 4 mg/kg ■ Bicuculline 2 mg/kg + Muscimol 4 mg/kg, ◆ 0,9% NaCl (control). Significant differences: *P < 0,05, **P < 0,01.

Effects of diazepam on core body temperature in rats after systemic application

Systemic administration of diazepam in dose 1 mg/kg does not produce significantly changes in core body temperature in rats (**Fig. 2**). Intraperitoneal administration of diazepam in

dose of 2.5 or 5 mg/kg caused fall in core body temperature in rats at 30, and 60 min after drug injection ($P < 0.01$), with a maximum observed at 30 min (**Fig. 2**). The control group treated with saline showed no change in body temperature of the rats.

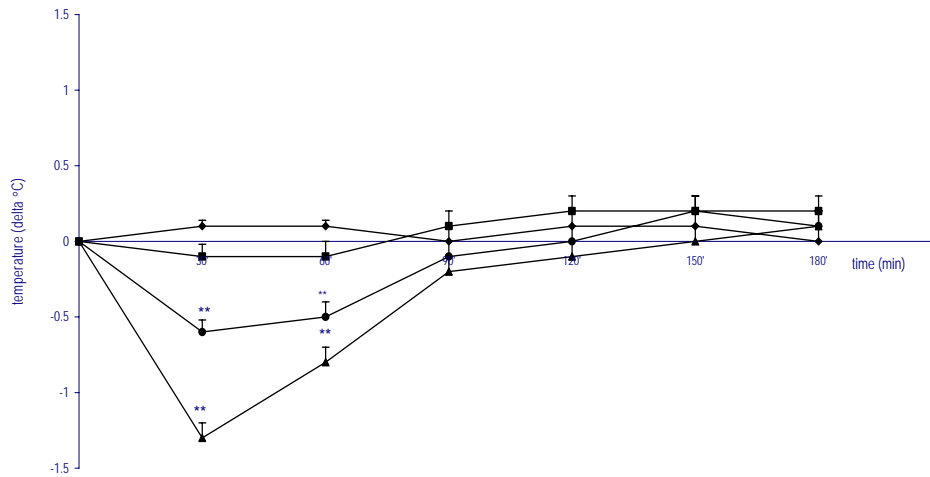


Fig. 2. Effects of intraperitoneal application (i.p.) of diazepam on body temperature in rats. Mean change (temperature delta °C) after i.p. administration of: ■ diazepam 1 mg/kg, ● diazepam 2,5 mg/kg, ▲ diazepam 5 mg/kg, and ◆ 0,9% NaCl (control). Significant differences: ** $P < 0,01$.

Hypothermia induced by diazepam (5 mg/kg i.p.) was antagonized with flumazenil (5 mg/kg i.p.) in pretreated rats (**Fig. 3**).

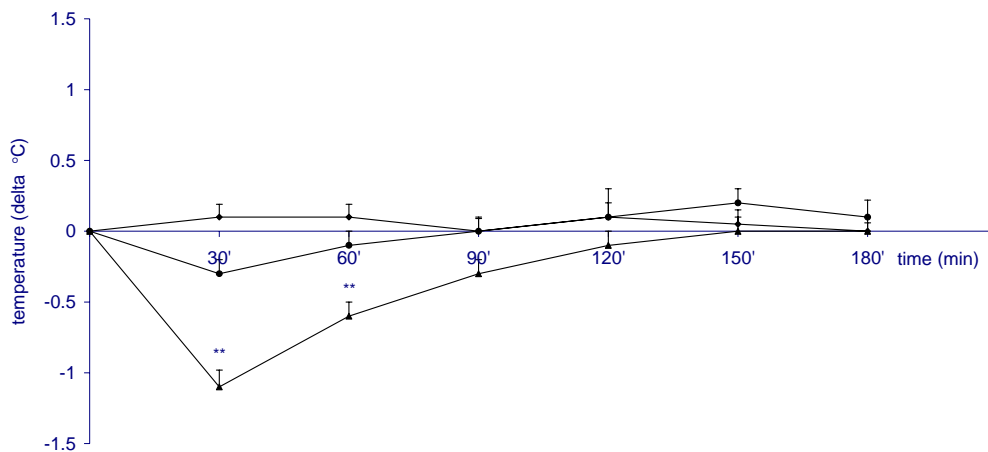


Fig. 3. Effect of intraperitoneal application of diazepam and flumazenil on body temperature in rats. Mean change (temperature delta °C) after i.p. administration of: ▲ diazepam 5 mg/kg, ■ flumazenil 5 mg/kg + diazepam 5 mg/kg, and ◆ 0,9% NaCl (control). Significant differences: * $P < 0,05$, ** $P < 0,01$.

DISCUSSION

GABA is the principal inhibitory neurotransmitter, which is widely distributed throughout the mammalian brain including hypothalamus (11). GABA interacts with two major types of receptors, the ionotropic GABA_A, and metabotropic GABA_B receptors. (12). Probably, the hypothermic response induced by GABA-ergic agents which act as direct GABA agonists is mediated by stimulation of GABA_A and GABA_B receptors (13). In mechanisms of GABA-mediated hypothermia may be involved other central neurotransmitters including serotonin, acetylcholine, dopamine (14-15). Experimental studies reported interactions between GABA-ergic and opioid system in rat thermoregulation (16).

Results in the present study show dose-dependent hypothermia induced by intraperitoneal injection of muscimol, a selective GABA_A agonist or diazepam, a positive modulator of GABA_A receptors, in rats. These findings might indicate involvement of direct receptor mechanism in hypothermia produced by substances with GABA-mimetic action.

Pretreatment of the rats with bicuculline, a selective GABA_A antagonist or flumazenil, a competitive benzodiazepine receptor antagonist, blocked the hypothermic response produced by muscimol or diazepam, respectively. These results suggest that hypothermic effect of muscimol or diazepam is a receptor-specific. It has been reported that hypothermia induced by diazepam was decreased in animals pretreated with high dose of bicuculline and picrotoxin, while hypothermic effect of diazepam was potentiated with low dose of bicuculline (17).

The hypothermic effects of muscimol or diazepam described in the present study may be clarified as regulated hypothermia. According to Gordon (18) neurochemical-induced decrease in core body temperature can be defined as unresisted or regulated hypothermia.

Our results indicate the role of GABA_A-receptor mechanism in hypothermic response induced by muscimol or diazepam.

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- NIKOLOV R., et al.*
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