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European Journal of Pharmacology 426 (2001) 179–183

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NMDA receptors are involved in dithiothreitol-induced hypothermia

Frédéric Canini *, Thomas Bréjot, Philippe d'Aléo, Sarah Mercier, Lionel Bourdon

Centre de Recherches du Service de Santé des Armées, Département des Facteurs Humains, 24 Avenue des Maquis du Grésivaudan, BP 87, F-38702 La Tronche Cedex, France

Received 15 March 2001; received in revised form 10 July 2001; accepted 13 July 2001

Abstract

Sulfhydryl-reducing agents, such as dithiothreitol, modulate glutamate *N*-methyl-D-aspartate (NMDA) receptors. Since these receptors are involved in thermoregulatory processes, we studied the effects of their positive modulation, through a dithiothreitol-induced reduction of the receptor redox site, on thermoregulation in rats maintained at an ambient temperature of 20–22 °C. Given intraperitoneally at the dose of 25 and 50 $mg \cdot kg^{-1}$, dithiothreitol induced dose-dependent hypothermia. The prior administration of 0.5 $mg \cdot kg^{-1}$ of (±)-dizocilpine maleate (MK801), a non-competitive glutamate NMDA receptor antagonist, blocked most of the dithiothreitol-induced hypothermia. MK801 given alone was followed by slight transient hyperthermia. This confirms the involvement of NMDA receptors in thermoregulation and suggests that they might be under redox modulation.

Keywords: Dithiothreitol; NMDA receptor; Hypothermia; Redox

1. Introduction

The glutamate *N*-methyl-D-aspartate (NMDA) receptor is a key receptor in the excitatory neurotransmission. NMDA function, modulated by endogenous products, appears to be highly sensitive to the oxidizing potential of the extracellular environment (Dingledine et al., 1999). Some reductants, such as ascorbate, act as inhibitors of NMDA receptors, while others, such as dithiothreitol, act as activators (Majewska et al., 1990). In vitro studies have shown that dithiothreitol, a sulfhydryl-reducing agent, enhances the NMDA-induced electrophysiological response (Aizenman et al., 1989) and NMDA-driven Ca^{2+} fluxes (Majewska et al., 1990; Reynolds et al., 1990) by increasing the opening frequency of the receptor (Tang and Aizenman, 1993). This positive modulation of glutamate NMDA receptors may have consequences on the physiological functions in which these receptors are involved. For example, sulfhydryl-reducing agents increase the NMDA-triggered release of catecholamines (Woodward, 1994; Woodward and Blair, 1991), restore the NMDA-induced long-term potentiation previously decreased by oxidizing drugs (Gozlan et al., 1994, 1995) and enhance the NMDA-induced nociceptive behavior (Laughlin et al., 1998).

Several facts favor the involvement of NMDA receptors in thermoregulatory function. In anesthetized rats, in situ injections of glutamate into the hypothalamic ventromedial nucleus are followed by an increase in sympathetic nerve activity in brown adipose tissue (Yoshimatsu et al., 1993). This agrees with the dramatic increase in brain and rectal temperature observed in anesthetized rats after an intracerebroventricular injection of NMDA (Hara et al., 1996, 1997). In situ injections of glutamate into the dorsomedial hypothalamus reduce the thermogenic activity, while injections into the medial preoptic area lead to a biphasic response with a decrease and then an increase in heat production (Yoshimatsu et al., 1993). However, the overall effect of NMDA activation in awake rats is rather hypothermic, since hyperthermia is observed after acute inhibition of the NMDA receptor by a peripheral injection of (±)-dizocilpine maleate (MK801) (Pechnick et al., 1989), a glutamate NMDA receptor non-competitive antagonist (Wong and Kemp, 1991). The hypothermic activity of the NMDA receptors noticed in awake rats maintained at ambient temperature (T_{db}) between 20 and 22 °C should therefore be enhanced by the positive modulation of NMDA receptors by the sulfhydryl-reducing agent dithiothreitol. Furthermore, MK801 should suppress the activating effect of dithiothreitol since MK801 blocks the NMDA receptor channel in a non-competitive manner, whereas dithio-

* Corresponding author. Tel.: +33-4-76-63-69-50; fax: +33-4-76-63-69-45.

E-mail address: caninif@compuserve.com (F. Canini).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01219-5

threitol increases its opening frequency (Tang and Aizenman, 1993). Furthermore, we administered MK801 before saline or dithiothreitol in order to have a similar channel-blocking effect because dithiothreitol may modify MK801 binding (Reynolds et al., 1990).

2. Materials and methods

2.1. Animals

Thirty-six male OFA Sprague–Dawley rats (Iffa Credo, Les Oncins, France), weighing 400–450 g at the beginning of the investigation, were used. The rats were housed at three per cage ($26 \times 40 \times 15$ cm) in an air-conditioned room with a controlled ambient temperature ($T_{db} = 22 \pm 1$ °C), relative humidity (r.h. = 40–50%) and light (12/12-h light/dark schedule with lights on at 7 a.m.). Food and water were available ad libitum except during the experimental sessions. The rats were allowed to adapt to laboratory conditions for at least 7 days prior to the beginning of the investigations. During this period, they were weighed and manipulated every morning to get them used to being handled. The experiment was approved by the Institutional Ethics Committee for Animal Care.

2.2. Drugs

Dithiothreitol (Sigma, Saint-Quentin-Fallavier, France) was administered intraperitoneally (i.p.) at the doses of 25 and 50 $mg \cdot kg^{-1}$. In order to have the same volume injected, the drug was freshly dissolved in 0.9% sterile saline at the respective concentration of 25 and 50 $mg \cdot ml^{-1}$. (±)-Dizocilpine maleate (MK801, Sigma) was administered i.p. at the dose of 0.5 $mg \cdot kg^{-1}$ after being dissolved in 0.9% sterile saline at the concentration of 0.5 $mg \cdot ml^{-1}$. The same volume was used for sterile saline injections.

2.3. Experimental design

The experiment was carried out in 36 rats submitted to two successive investigations with a 10-day interval. In the first investigation, the rats were randomly distributed into one of three groups, each containing 12 rats, according to their treatment: 0.9% sterile saline (saline, $n = 12$), 25 $mg \cdot kg^{-1}$ of dithiothreitol (dithiothreitol-25, $n = 12$) and 50 $mg\ kg^{-1}$ of dithiothreitol (dithiothreitol-50, $n = 12$). At 8 a.m., the rats were weighed and placed in individual experimental cages ($26 \times 40 \times 15$ cm) in their housing room for a 1-h period to become accustomed to the novel condition. The cages were then carried into the laboratory room (ambient temperature: $T_{db} = 20–22$ °C) for the investigation. Measures consisted of nine successive colonic temperature (T_{co}) measurements, separated by 30 min. After the third T_{co} measurement, the rats received i.p. either dithiothreitol or the same volume of saline, dividing the investigation into two periods: a 90-min reference period (three measurements) and a 180-min treatment period (six measurements). At the end of the investigation, the rats were weighed again and put back into their home cages.

An interval of 10 days was believed to be sufficient as a withdrawal period, in respect of the rapid reversibility of hypothermia in our dithiothreitol-25 group in the first investigation (see Results, Fig. 1). In the second investigation, three rats from each first investigation group were taken to form groups of nine rats. Each new group was then named according to the drug to be received: 0.9% sterile saline (saline, $n = 9$), 0.5 $mg\ kg^{-1}$ MK801 (MK801, $n = 9$), 50 $mg \cdot kg^{-1}$ dithiothreitol (dithiothreitol, $n = 9$) and 0.5 $mg \cdot kg^{-1}$ MK801 + 50 $mg \cdot kg^{-1}$ dithiothreitol (MK801 + dithiothreitol, $n = 9$). At 8 a.m., the rats were weighed and then placed in their experimental cages in the housing room for 1 h. Once again, they were moved to the laboratory room ($T_{db} = 20–22$ °C). This investigation consisted of 11 successive T_{co} measurements. After three T_{co} measurements, the rats received i.p. either 0.5 $mg \cdot kg^{-1}$ MK801 (MK801 and MK801 + dithiothreitol groups) or the same volume of saline (saline or dithiothreitol groups). After two other T_{co} measurements, dithiothreitol (dithiothreitol and MK801 + dithiothreitol groups) or saline (saline and MK801 groups) was injected i.p. The investigation was thus divided into three time periods: a 90-min reference phase (three measurements), a 60-min MK801 treatment phase (two measurements) and a 180-min full treatment phase (six measurements). After the investigation, the rats were weighed again and put back into their home cages.

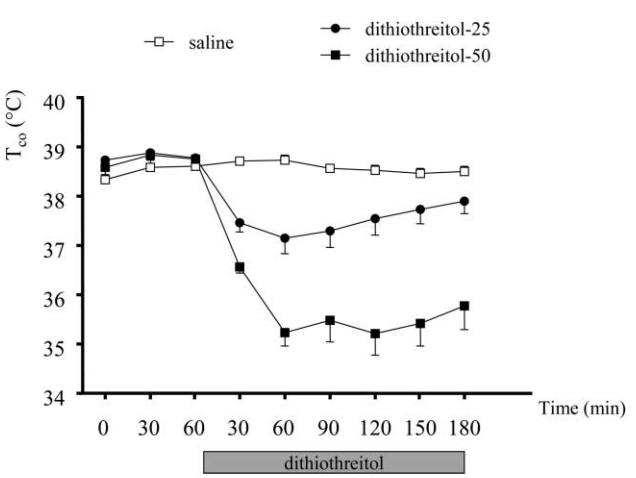

Fig. 1. Time course of changes in colonic temperature (T_{co}, °C) in rats treated with saline (saline, $n = 12$), 25 $mg \cdot kg^{-1}$ of dithiothreitol (dithiothreitol-25, $n = 12$) and 50 $mg \cdot kg^{-1}$ of dithiothreitol (dithiothreitol-50, $n = 12$). The reference period is represented by the first three T_{co} measurements. Treatment was given just after the third measurement. Thereafter, six T_{co} measurements at 30-min intervals were taken, corresponding to the treatment period. Experimental points are expressed as means ± S.E.M.

2.4. Physiological measurement

Colonic temperature was measured using a calibrated Cu/Ct thermocouple inserted into the colon at 7 cm beyond the anal margin. Calibration was done in a thermostated water bath. Temperature was measured in rats in their experimental cages. The animals were handled as little as possible. T_{co} was assessed at a rate of 12 values per min and data were read in real time on a computer screen. The T_{co} measurement ended when a plateau was reached. After the investigation, each T_{co} measurement was analyzed in order to determine its duration as well as the duration and the mean value of the plateau, which represented the final T_{co} value. The basal T_{co} value was the average of the first three measurements.

2.5. Statistical analysis

The statistical analysis was done using factorial analysis of variance (ANOVA) with one factor at three levels (Treatment) in the first investigation, and two factors at two levels in the second (Treatment MK801, Treatment dithiothreitol). The time course of T_{co} was analyzed using ANOVA for repeated measures with a treatment effect at three (first investigation) and four levels (second investigation). If necessary, Bonferroni–Dunn post-hoc tests for all couples were carried out. The significance level was set at $P < 0.05$. The data are expressed as means ± standard error of the mean (S.E.M.).

3. Results

The duration of each T_{co} measurement was in the same range in both investigations (first investigation: 84 ± 1 s, second investigation: 90 ± 1 s). No statistical difference in T_{co} measurement duration was observed among treatment groups in each investigation. The duration of the T_{co} plateau was similar between investigations (first investigation: 21 ± 1 s, second investigation: 20 ± 1 s, ns) and among treatment groups in each investigation.

In the reference period of the first investigation (Fig. 1), the rats exhibited similar basal T_{co} values: saline: 38.5 ± 0.1 °C; dithiothreitol-25: 38.8 ± 0.1 °C; dithiothreitol-50: 38.7 ± 0.1 °C. After dithiothreitol injection, T_{co} decreased dose-dependently (Repetition: $P < 0.001$ and Treatment: $P < 0.001$ with dithiothreitol-50 vs. saline: $P < 0.001$ and dithiothreitol-50 vs. dithiothreitol-25: $P < 0.001$). The lowest T_{co} values were reached 60 min after the injection (saline: 38.7 ± 0.1 °C; dithiothreitol-25: 37.1 ± 0.3 °C; dithiothreitol-50: 35.2 ± 0.3 °C; Treatment: $P < 0.001$).

In the reference period of the second investigation (Fig. 2), the same basal T_{co} values were measured in rats of the different treatment groups: saline: 38.6 ± 0.1 °C; MK801: 38.6 ± 0.1 °C; dithiothreitol: 38.5 ± 0.2 °C and MK801 + dithiothreitol: 38.6 ± 0.1 °C. T_{co} values did not differ from those of the first investigation. Moreover, no difference among the rats of the saline ($n = 3$), dithiothreitol-25 ($n = 3$) and dithiothreitol-50 ($n = 3$) groups of the first investigation was noticed in each of the treatment groups of the second investigation. MK801 administration was followed by slight hyperthermia 30 min after the injection (Treatment MK801, $P < 0.01$), but not after a further 30 min. The injection of dithiothreitol was followed by hypothermia only in rats previously treated with saline (Repetition: $P < 0.001$ and Treatment: $P < 0.001$ with dithiothreitol vs. saline: $P < 0.001$; dithiothreitol vs. MK801: $P < 0.001$ and dithiothreitol vs. MK801 + dithiothreitol: $P < 0.001$). In the dithiothreitol group, the lowest T_{co} was observed 60 min after the injection and was similar to that measured in the first investigation. The dithiothreitol-induced hypothermia was potently diminished by the prior administration of MK801 (saline: 38.8 ± 0.1 °C; MK801: 38.8 ± 0.1 °C; dithiothreitol: 35.2 ± 0.3 °C; MK801 + dithiothreitol: 37.6 ± 0.1 °C; Treatment MK801: $P < 0.001$; Treatment dithiothreitol: $P < 0.001$ and Interaction: $P < 0.001$). Indeed, 70% of the hypothermic effect induced by dithiothreitol was abolished by the prior administration of MK801 (dithiothreitol: −3.3 °C, MK801 + dithiothreitol: −1.0 °C).

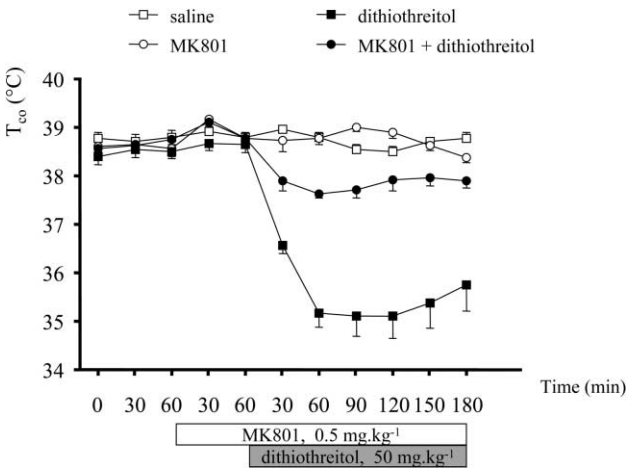

Fig. 2. Time course of changes in colonic temperature (T_{co}, °C) in rats treated successively with saline/saline (saline, $n = 9$), saline/50 mg·kg^{-1} of dithiothreitol (dithiothreitol, $n = 9$), 0.5 mg·kg^{-1} of MK801/saline (MK801, $n = 9$) and 0.5 mg·kg^{-1} of MK801/50 mg·kg^{-1} of dithiothreitol (MK801 + dithiothreitol, $n = 9$). The reference period is represented by the first three T_{co} measurements. MK801 treatment was given just after the third measurement. The MK801 treatment period concerned the 4th and the 5th points. Dithiothreitol treatment was given just after the 5th point; the full treatment period corresponded to the next six T_{co} measurements. Experimental points are expressed as means ± S.E.M.

4. Discussion

The main findings of this study were that (i) in rats maintained at an ambient temperature of $T_{db} = 20–22$ °C,

the sulfhydryl-reducing agent dithiothreitol induced hypothermia, whose extent and duration were dose-dependent; (ii) the dithiothreitol-induced hypothermia was blocked by MK801, a non-competitive channel blocker of the glutamate NMDA receptor (Wong and Kemp, 1991).

Dithiothreitol is a non-specific sulfhydryl-reducing agent. It may act at numerous levels in the thermoregulatory processes leading to hypothermia, with NMDA receptors being among its potential sites of action. However, three hypotheses can be discussed. Indeed, dithiothreitol may induce hypothermia through a biochemical effect at a level other than that of the NMDA receptor since it has been shown that dithiothreitol acts at the level of the protein of phosphoinositide transduction (Vignes et al., 1992) on G-protein-coupled receptors (Malbon et al., 1987), on 5-HT_{1A} receptors (Emerit et al., 1991), on β-adrenoceptors (Pedersen and Ross, 1985), on substance P receptors (Sharma and Musacchio, 1987) and on acetylcholine receptors (Rojas et al., 1991). In such a case, the blocking effect of MK801 strongly suggests that NMDA receptors are involved somewhere in the process of dithiothreitol-induced hypothermia. Dithiothreitol may also induce hypothermia by acting directly at the level of the NMDA receptors. Evidence from the literature suggests that dithiothreitol may modify the activity of NMDA receptors through a disulfide-reducing action. Indeed, the NMDA-induced enhancement in nociceptive behavior is exaggerated by dithiothreitol. Such an effect is blocked by an oxidizing drug (Laughlin et al., 1998). Similar observations are reported at the level of cell functions involving NMDA receptors, such as the NMDA-induced release of neurotransmitters (Woodward and Blair, 1991) or NMDA-triggered Ca^{2+} fluxes (Reynolds et al., 1990). A third hypothesis is that dithiothreitol produces the hypothermia by acting simultaneously at several levels, including NMDA receptors.

The observation that the blocking effect of MK801 on dithiothreitol-induced hypothermia was not complete also deserves discussion. The defect in the blocking effect of MK801 may be attributed to the activity of dithiothreitol on a pathway that does not involve NMDA receptors. That an insufficient number of NMDA receptors were blocked should also be considered. Dithiothreitol may increase NMDA receptor function only for those receptors in which the channel remains free of MK801, inasmuch as MK801 uncompetitively blocks the permeability of the channel (Wong and Kemp, 1991) and dithiothreitol acts by enhancing the opening frequency of the channel (Tang and Aizenman, 1993). Moreover, the inhibition provided by MK801 should have lasted throughout the whole investigation since the MK801-induced inhibition of the NMDA receptor lasts at least 3 h (Wong et al., 1986).

However, the hypothesis that the hypothermic action of dithiothreitol comes from a modulation of NMDA receptors should be studied further. In our study carried out with awake rats maintained at $T_{db} = 20–22$ °C, the mean T_{co} observed during the reference period was similar in all treatment groups. The T_{co} values can be explained by the deep location of the T_{co} probe, which thus measures the highest body temperature (Romanovsky et al., 1997) and the duration of the investigation, because the rats were out of their home cages for more than 60 min (Briese and de Quijada, 1970). Under these conditions, dithiothreitol induces hypothermia while the inhibition of the NMDA receptor by MK801 produces transient slight hyperthermia. These results were similar to those of other studies showing dithiothreitol-induced hypothermia in rabbits (Riedel and Maulik, 1999) and MK801-induced hyperthermia in rats (Pechnick and Hiramatsu, 1994; Pechnick et al., 1989; Pucilowski et al., 1991). The observation that dithiothreitol induced a deep hypothermia while MK801 produced a slight hyperthermia strengthens the hypothesis that NMDA receptors are activated by dithiothreitol. It also suggests that, under basal conditions, NMDA receptors are in a down-activated state and that the disulfide bonds within NMDA receptors are in an oxidized state. This hypothesis is in agreement with the results of studies of NMDA-induced nociceptive behavior: oxidizing drugs do not modify the occurrence of NMDA-induced nociceptive behavior while dithiothreitol mainly increases their frequency (Laughlin et al., 1998). In rabbits, dithiothreitol induces an increase in vasodilation and panting together with a decrease in O_2 consumption (Riedel and Maulik, 1999), suggesting a coordinated hypothermic reaction. In this case, the NMDA receptors modulated by dithiothreitol are probably not located within the ventromedial hypothalamus, where a glutamate injection increases heat production, but rather at the level of the medial preoptic area or the dorsomedial hypothalamus, where glutamate injection produces a decrease in heat production (Yoshimatsu et al., 1993).

In conclusion, dithiothreitol administration provokes dose-dependent hypothermia which is blocked by a prior administration of MK801, while MK801 alone induces a slight hyperthermia. This suggests that NMDA receptors are implicated in thermoregulatory processes and that modulation of their redox state might be involved in thermophysiological function.

Acknowledgements

We are grateful to Alain Buguet for his helpful comments and to David Popieul for his skilled technical help and animal care. This work was supported by grants from the Service de Santé des Armées.

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