Pharmacology Biochemistry and Behavior, Vol. 59, No. 1, pp. 233–237, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00337-7

Effects of Diethylenetriamine on NMDA-Induced Increase of Blood Pressure in Rats

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Received 3 December 1996; Revised 11 May 1997; Accepted 28 May 1997

MAIONE, S., M. PALLOTTA, J. LEYVA, E. PALAZZO AND F. ROSSI. *Effects of diethylenetriamine on NMDA-induced increase of blood pressure in rats.* PHARMACOL BIOCHEM BEHAV **59**(1) 233–237, 1998.—We investigated the effects of three doses of diethylenetriamine (DET; 0.1–1–10 nmol/rat), putative ligand at the polyamine site on NMDA receptors, on blood pressure increase induced by *N*-methyl-D-aspartate (NMDA, 2 nmol/rat) microinjected at the periaqueductal gray (PAG) matter. Said doses of DET did not modify basal arterial blood pressure. Pretreatment with DET, depending on the dose used, either potentiated (DET, 0.1 nmol/rat), reduced (DET, 1 nmol/rat) the NMDA effects or left them unchanged (DET, 10 nmol/rat). DET 10 nmol/rat, microinjected 5 min before spermidine (SPD, 0.02–2 nmol/rat), significantly antagonized SPD modulation on the NMDA-induced pressor changes. These data, in agreement with the functional findings in vitro, suggest that also in vivo DET has pharmacological activities quite different from a pure antagonist but shows multiple actions on the NMDA receptors. © 1998 Elsevier Science Inc.

Glutamate receptors Polyamines Pressor neurons Rat

GLUTAMATE *N*-methyl-D-aspartate (NMDA) subtype receptors are ligand-gated ion channels that mediate excitatory neurotransmission in the central nervous system (9). Due to necessary copresence of glutamate and glycine for the gating of these receptor channels, both amino acids are currently referred to as NMDA receptors coagonists. Nevertheless, several other endogenous factors, including the polyamines spermine and spermidine (12), seem to be involved in the allosteric modulation of NMDA receptors activities (1,5,7). In vitro reports have shown that both spermine and spermidine, at concentrations ranging from 10 to 300 μM, increase the frequency of NMDA receptors channel opening and also potentiate the ionic current across them (15). Millimolar concentrations of these polyamines, however, negatively modulate NMDA receptor functions. Little is known about the role of the polyamine modulator sites on the NMDA receptor as, in contrast to glycine site, there are few selective antagonists available (6). Some drugs have been proposed as polyamine antagonists and, among these, ifenprodil (13), arcaine and, more recently, the long chain diamines 1,10 diaminodecane (DA10) and 1,12 diaminododecane (DA12) (17,22). These decreased [^{3}H]MK 801 binding at the NMDA channels and produced a voltage-dependent NMDA receptors flickery block (16,19). Subsequently, Benveniste et al. (3) confirmed that DA10 blocks NMDA receptors by an open-channel mechanism. They also observed that diethylenetriamine (DET) has an inhibitory effect on NMDA receptors that contrasts with data obtained from radioligand binding studies that indicates that DET is a polyamine antagonist (22). Robichaud et al. (14), in vitro, and Doyle et al. (4), in vivo, showed that spermine potentiated NMDA-induced epileptiform discharges. In particular, Robichaud et al. demonstrated that in rat cortical wedge the spermine-induced effect was not antagonized by DET. However, NMDA-stimulated release of rat hippocampal [^{3}H]norepinephrine has been shown to be inhibited by DET (23).

Overall, these in vitro studies demonstrate that the pharmacological profile of DET remains unknown. In same cases it seems to be a noncompetitive NMDA receptor antagonist, but in others it lacks any effect. However, there is convincing evidence that this drug is not a selective antagonist of the fa-

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cilitator polyamine NMDA receptors. To further clarify the in vivo pharmacological effect of DET on NMDA receptors, we carried out this study at the level of the pressor neurons within periaqueductal gray (PAG) matter of anesthetized rats.

METHOD

Subjects

Male Sprague–Dawley rats, weighing 260–320 g, were housed three per cage and kept in a controlled environment with constant temperature (21 ± 1°C) and relative humidity (60%), under a regular light/dark schedule (lights 07.00–19.00 h). Food and water were always available. Animal care was in compliance with Italian (D.M. 116/92) and EEC (O.J. of E.C. L358/1 18/12/1986) regulations on protection of laboratory animals.

Surgical Preparation and Treatment

Surgical procedure. On the day of the experiment, each animal was anesthetized with urethane (1.2 g/kg, IP). Because the duration of the experiments was less than 3 h, we chose this anesthetic because its effect lasts for at least 2–3 h and also based on past experience (8). A polyethylene catheter (PE 50) was placed in a femoral artery for direct measurement of arterial blood pressure. A pressure transducer (model 52-9966), displayed on a Harvard Universal oscillograph 50-9323 (Harvard Apparatus Limited, Edenbridge, UK) was used. After the trachea was cannulated, the animal was artificially ventilated with a rodent respirator (Harvard 683) using a one-to-one mixture of room air and 100% O_2. Gallamine triethiodide (10 mg/kg, IV) was administered for muscular paralysis, and supplemented (5 mg/kg, IV) whenever the animal exhibited spontaneous respiratory motions. Due to the PAG involvement on modulation of respiration (2,10), the cardiovascular response recorded after PAG stimulation may be significantly affected by concurrent changes on respiratory activity. Hence, in this study the animals needed to be paralyzed. We periodically (10–15 min) tested the level of anaesthesia during the course of the experiment by touching the cornea with a spatula. The possible presence of neuroreflex indicated the need for an additional amount of urethane (0.25 g/kg IP). Immediately after surgery, each rat was placed on a homeothermic temperature-control blanket (Harvard Apparatus Limited, Edenbridge, UK) with no recording of the core temperature of the animal during the experiment. The head was secured to a stereotaxic frame (David Kopf Instruments, Tujunga, CA) for the direct intracerebral administration of *N*-methyl-D-aspartic acid (NMDA, 0.07–7 nmol/rat), DL-2-amino-5-phosphonovaleric acid (APV, 5 nmol/rat), spermidine (SPD, 0.02–2 nmol/rat), putrescine (PTS, 0.02–2 nmol/rat), and diethylenetriamine (DET, 0.1–10 nmol/rat) into the dorsal–lateral PAG matter. The intracerebral microinjections were carried out with multibarrel glass micropipettes (FHC Brunswick, ME) with an outside tip diameter of 40–50 $<\mu$m pulled by a vertical pipette puller (David Kopf Instruments, Tujunga, CA). Injections were carried out by a pressure system: micropipettes were carried in a stereotaxic micromanipulator (David Kopf Instruments) and connected by a polyethylene PE 20 tubing to a Hamilton microsyringe that was connected to an infusion pump (Burco Instruments, Sharon, CT; mod. STC100). Coordinates from the Paxinos and Watson atlas (11) (measured in mm from bregma AP: −7.8; L: 0.5; V: 4.5) were used. A control volume of 50 nl of saline or the same volume of drug solution was injected over a period of 5 s. To exclude any fluid microinjection artefacts, all rats received an initial injection of 50 nl saline into the PAG. Experiments were carried out only in those rats with stable blood pressure values (mean values during the experiment = 95 ± 10 mmHg; level of fluctuation = 11 ± 5 mmHg). Blood pressure values recorded 2 and 5 min before drug injections were used as control.

The first group of animals received three doses of NMDA (0.2, 2, and 7 nmol/rat) at 30–40 min intervals. The second group was treated with APV 5 nmol/rat alone or in combination, 5 min before, with NMDA 7 nmol/rat. To measure the effect of DET on NMDA-induced arterial hypertension, the third group of rats received either 0.1, 1, or 10 nmol DET 5 min before 2 nmol NMDA treatment. Due to NMDA excitotoxicity, we injected the dose of 2 nmol/rat instead of 7 nmol/rat. This allowed us to administer more that one time NMDA in the same animal. Based on previous experience, we avoid to inject again and again into the PAG doses of NMDA higher than 4 nmol/rat. In fact, these can induce severe cardiorespiratory effects and also may kill the animal. Similarly, the effect of SPD or PTS on NMDA-induced arterial hypertension was measured by administering either 0.02, 0.2, or 2 nmol SPD or PTS to rats of the fourth group 5 min before they received 2 nmol NMDA. Each rat was treated with only one dose of either DET or SPD before NMDA injection. To further test whether DET 10 nmol also affected NMDA-induced hypertension another group of rats was used. Each rat was treated with DET 10 nmol 6 s, 2 min, and 5 min prior to administration of NMDA 2 nmol.

After each experiment, the stereotaxic coordinates at the injection sites were checked histologically. A volume of 50 nl of methylene blue (0.2%) was injected intracerebrally 5 min before killing the rat with a high dose of pentobarbital (200 mg/kg IV). The animal was perfused intracardially with 50 ml of PBS followed by 50 ml of a 10% formalin solution in PBS. Brain was removed and immersed into saturated formalin solution for 24 h; the injection site was ascertained by using two consecutive sections (40 μm), one stained with cresyl violet to identify nuclei, and the other one unstained to determine dye diffusion. Only those rats whose microinjected site was located within the dorsal–lateral PAG area were used for data computation. The rat PAG represents a relatively large mesencephalic area with an horizontal axis of about 1.95 mm and a vertical axis of about 2.25 mm. It is currently recognized that the theoretical radius of the sphere of 1 μl volume is about 1.240 mm, indicating that our injections (only 50 nl of volume) were made inside the PAG, as confirmed histologically.

Drugs

N-methyl-D-aspartic acid (NMDA), DL-2-amino-5-phosphonovaleric acid (APV), spermidine phosphate salt (SPD), putrescine (PTS), gallamine triethiodide, pentobarbital, and urethane were obtained from Sigma Chemical Co. (St. Louis, MO), and diethylenetriamine (DET) from Aldrich-Chemie (Steinheim, Germany).

Statistics

All results are expressed as mean ± standard error (SE), with $p < 0.05$ considered as the level of significance. The statistical analysis of the cardiovascular changes was performed by one-way analysis of variance (ANOVA), followed by the Newman–Keuls test for paired groups (19).

RESULTS

Injection of 50 nl saline into the dorsal lateral PAG area of anesthetized rats neither modified basal systolic arterial blood

TABLE 1

MAXIMUM BLOOD PRESSURE CHANGES INDUCED BY MICROINJECTIONS OF THE FOLLOWING DRUGS INTO THE PERIAQUEDUCTAL GRAY (PAG) MATTER OF ANAESTHETIZED RATS

Treatment	Dose (nmol/rat)	*n*	Maximum Blood Pressure Increase (mmHg ± SE)
Saline	50 nl	8	3 ± 0.9
NMDA	0.2	7	11.3 ± 3*
NMDA	2	8	18.6 ± 5*
NMDA	7	8	36.3 ± 8†
APV	5	5	3.5 ± 1
APV + NMDA	5 + 7	6	8.2 ± 4‡
SPD	0.02	5	2.5 ± 0.5
SPD	0.2	5	3 ± 1.8
SPD	2	5	3.5 ± 2
PTS	0.02	6	5.2 ± 2.6
PTS	0.2	7	4.7 ± 1.9
PTS	2	7	3.7 ± 1.8
PTS + NMDA	0.02 + 2	5	15.6 ± 3.8*
PTS + NMDA	0.2 + 2	6	13.5 ± 2.9*
PTS + NMDA	2 + 2	6	17.8 ± 3.5*

Changes in systolic arterial blood pressure (ΔABP) (mmHg ± SE) in anesthetized (urethane, 1.2 g/kg IP) rats after microinjections of saline, *N*-methyl-D-aspartate (NMDA; 0.07–7 nmol/rat), spermidine (SPD; 0.02, 0.2, or 2 nmol/rat) putrescine (PTS, 0.02, 0.2, or 2 nmol/rat) into the dorsal lateral periaqueductal gray (PAG) area. A group of rats treated with NMDA were pretreated or not, 5 min before, with APV (5 nmol/rat), selective antagonist of NMDA receptors, or PTS (0.2–2 nmol/rat). Data are shown as the mean ± SE (n = 5–8). Significant differences are shown by symbols (*$p < 0.05$, †$p < 0.01$ vs. saline; ‡$p < 0.01$ vs. NMDA 7) and have been determined by ANOVA followed by Newman-Keuls tests for paired groups.

pressure (97 ± 8 mmHg) nor NMDA-induced pressor changes (Table 1 and Fig. 1). NMDA (0.2–7 nmol/rat) administered into the same area significantly, $F(6, 7) = 1.95$, $p < 0.01$, increased arterial blood pressure in a dose-dependent manner [linear trend statistically significant: $F(6, 7) = 11.9$, $p < 0.008$; slope = 10.5; $r^2 = 0.57$] (Table 1). Cardiovascular changes occurred immediately (2–5 s) after administration of NMDA. The maximum pressor response was recorded within 2–3 min, and within 5–12 min the value returned to control depending on dosage. Pretreatment, which was done 5 min before NMDA (7 nmol/rat) using APV (5 nmol/rat), a selective antagonist of the NMDA receptors, significantly, $F(7, 5) = 1.68$, $p < 0.01$, reduced NMDA-induced pressor change (Table 1). To evaluate the possible effects of DET on the NMDA-induced cardiovascular changes we chose the NMDA hypertensive response induced by the 2 nmol/rat dose. DET by itself did not modify basal arterial blood pressure (data not shown), but interfered on NMDA-induced effect. DET lowest dose (0.1 nmol/rat) increased NMDA-induced hypertension, $F(7, 12) = 1.97$, $p < 0.05$, and the intermediate dose (1 nmol/rat) decreased the NMDA effect, $F(7, 9) = 2.48$, $p < 0.05$) (Fig. 1). The highest dose of DET (10 nmol/rat), microinjected 0.1 min before NMDA, only slightly and no significantly increased the NMDA effect (Fig. 2); however, DET 10 nmol/rat significantly, $F(7, 8) = 1.53$, $p < 0.05$, decreased the NMDA-induced increase in blood pressure when injected 2 min before NMDA (Fig. 2). The DET highest dose was ineffective when it was given 5 min before NMDA (Figs. 1 and 2). Moreover, this clearly ineffective dose of DET, significantly, $F(9, 12) = 1.29$, $p < 0.05$, inhibited the reduction of NMDA-induced pressor effect by 0.2 or 2 nmol SPD when administered into the PAG 5 min before SPD (Fig. 3). In agreement with our previous study (8) SPD lowest dose (0.02 nmol/rat) potentiated, $F(7, 9) = 2.07$, $p < 0.05$, NMDA-induced effects while higher doses (0.2–2 nmol/rat) decreased it, $F(5, 9) = 1.29$, $p < 0.05$ (Fig. 3). Unlike DET or SPD, PTS (0.02–2 nmol/rat) did not modify NMDA-induced effect (Table 1). DET, SPD, and PTS did not change basal arterial blood pressure (Table 1).

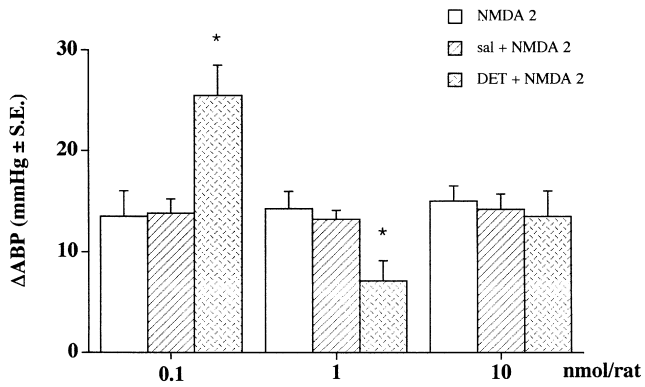

FIG. 1. Changes in arterial blood pressure (ΔABP) (mmHg ± SE) induced by NMDA (2 nmol/rat) before or after pretreatment with 50 nl saline (sal) or diethylenetriamine (DET, 0.1–10 nmol/rat) 5 min before NMDA. The drugs were administered into the dorsal lateral PAG area of anesthetized rats. Data are shown as the mean ± SEM (n = 8–13). Significant differences, determined by ANOVA followed by Newman–Keuls test, are marked with asterisks (*$p < 0.05$ respect to groups receiving NMDA + sal).

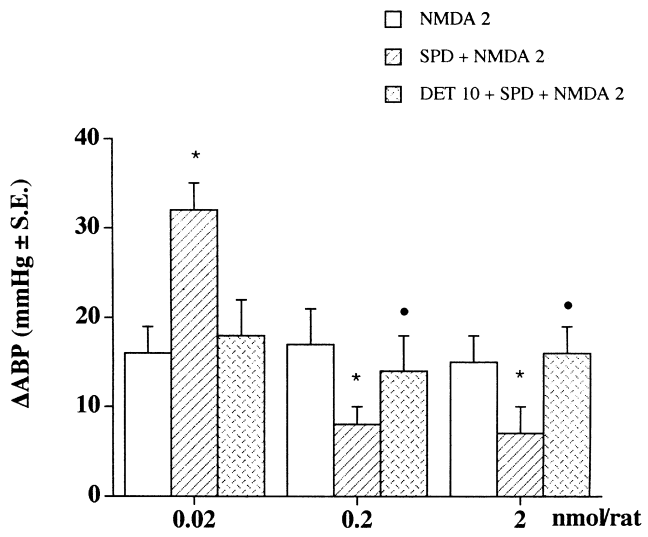

FIG. 2. Changes in arterial blood pressure (ΔABP) (mmHg ± SE) induced by NMDA (2 nmol/rat) before or after pretreatment with 50 nl saline (sal) or diethylenetriamine (DET, 10 nmol/rat) microinjected 0.1, 2, or 5 min before NMDA. The drugs were administered into the dorsal lateral PAG area of anesthetized rats. Data are shown as the mean ± SEM (n = 10–13). Significant difference, determined by ANOVA followed by Newman–Keuls test, is marked with an asterisk (*$p < 0.05$ respect to groups receiving NMDA + sal).

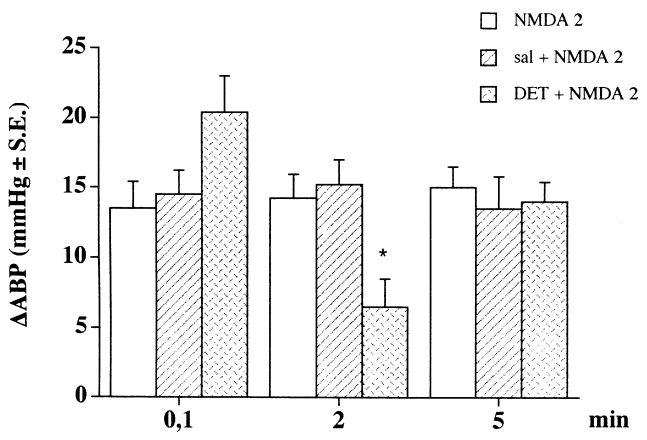

FIG. 3. Changes in arterial blood pressure (ΔABP) (mmHg ± SE) induced by NMDA (2 nmol/rat) before or after pretreatment with spermidine (SPD, 0.02–2 nmol/rat 5 min before NMDA) or SPD in combination with diethylenetriamine (DET, 10 nmol/rat 5 min before SPD). The drugs were administered into the dorsal lateral PAG area of anaesthetized rats. Data are shown as the mean ± SEM (n = 8–10). Significant differences, determined by ANOVA followed by Newman–Keuls test, are marked with asterisks (*$p < 0.05$ respect to groups treated with NMDA alone and *$p < 0.05$ respect to groups treated with NMDA + SPD).

DISCUSSION

Our results indicate that, depending on the dose used, DET can either potentiate, reduce the NMDA-induced cardiovascular effects, or leave them unchanged. As partially happens with spermine and spermidine, DET-modulated NMDA receptors in a biphasic concentration–response curve: DET lowest dose potentiated NMDA-induced pressor effect, but a reduction of this effect was observed when DET dose increased. However, DET pharmacological profile differed from SPD when the dose was further increased. We previously demonstrated (8), and now confirm, that by raising SPD dose, a block of NMDA-mediated effects occurs. Current in vivo research shows, however, that a higher dose of DET is devoid of any effects on the NMDA receptors in the PAG. On the other hand, the charged amine putrescine, which does not interact in vitro with NMDA receptors, did not modify NMDA-induced cardiovascular effects, and this further confirms that our model could be pharmacologically appropriate in evaluating the complex interaction of polyamines on NMDA receptors in vivo.

To date, some confusion seems to emerge from the in vitro studies about the action of DET. Studies on binding suggest a competitive partial agonist/antagonist interaction of DET with SPD-like drugs (17,22), NMDA-stimulated release of rat hippocampal [^{3}H]norepinephrine has been shown to be inhibited by DET (23), while electrophysiologically DET shows channel blocking properties (14,15). These effects are not complementary; hence, the difficulty in understanding the action of DET. This in vivo study shows that DET antagonized NMDA-induced cardiovascular effect only at a 1 nmol/rat dose. In agreement with the above studies, the blockade of NMDA receptors by DET may be secondary to the latter's interference with a negatively charged low-affinity polyamine site on the NMDA receptor channels. Subramaniam et al. (20) more recently argued that the blocking site of DET may correspond to a voltage-dependent anionic region close to the NMDA channel pore. This site seems to be different from both the facilitator polyamine site and a hypothetical voltage-independent hydrophobic diamine blocking site. Concerning the potentiation of the NMDA-induced effects with the lowest dose of DET, it is unlikely that this is due to an involvement of the facilitator polyamine site inasmuch as DET pretreatment only antagonized SPD's negative although positive modulatory effect on NMDA receptors. It could be suggested that in our in vivo model DET at the lowest dose would promote a partial removal of the tonic Mg^{2+}/Ca^{2+} block at the above-mentioned negatively charged surface on the mouth of the channel (18).

This study also demonstrated that the highest dose of DET (10 nmol/rat) was unable to modify NMDA-induced arterial hypertension, although it could prevent SPD negative modulation on NMDA receptors. For this reason, it is unlikely that DET may be metabolized, have another action at the high initial injection concentrations, or simply diffuse away from the injection site. Although there is no in vitro evidence in that a desensitization-like event takes place at the DET binding site, we tried to highlight the effect of the highest dose of DET on NMDA receptors administering DET 0.1 or 2 min before NMDA, as well as the usual 5 min. Decreasing the gap between DET 10 nmol/rat and NMDA injections, a biphasic effect of DET on NMDA-induced hypertension appeared. It is highly unlikely that an in vivo study, like this one, may throw further light on such a complex interaction, but we hope that our data coax other authors into further investigating this possibility.

Overall, this study confirms previous in vitro research that showed the complex pharmacological profile of DET. This study suggests that also in vivo, DET is pharmacologically different from a pure antagonist at the polyamine recognition site, but may have multiple actions on the NMDA receptors.

ACKNOWLEDGEMENTS

Financial support from MURST 40%, MURST 60%, and CNR, Italy, is gratefully acknowledged.

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