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MK-801 enhances noradrenergic neurotransmission in rat vas deferens

David Pubill *, Anna M. Canudas, Daniel Gasulla, Mercè Pallàs, Elena Escubedo, Jorge Camarasa

Laboratory of Pharmacology and Pharmacognosy, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain

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Abstract

The effect of MK-801 (dizocilpine) on the noradrenergic neurotransmission of the epididymal portion of rat vas deferens has been investigated. This drug potentiated the electrically induced responses (46.6% ± 5.09 at a concentration of 3.7 μM) and the contractile effect of exogenous noradrenaline with a concentration-dependent reduction of EC_{50} (from 0.99 ± 0.11 μM to 0.06 ± 0.01 μM). Moreover, MK-801 alone induced spontaneous contractile responses that were abolished by prazosin, not reversed by *N*-methyl-D-aspartate (NMDA) + glycine and that did not appear in organs obtained from reserpinized rats. In addition, MK-801 inhibited the [^{3}H]noradrenaline uptake in slices from rat vas deferens (IC_{50} = 1.79 ± 0.06 μM). Since these effects took place in the presence of magnesium and were sodium-dependent, a direct participation of the NMDA receptor complex can be ruled out, pointing to the inhibition of the cathecolamine uptake systems in the postganglionic sympathetic nerve endings as the most feasible mechanism.

Keywords: MK-801 dizocilpine; Vas deferens, rat; Noradrenaline; Uptake

1. Introduction

There is now extensive evidence that glutamic acid acts as a neurotransmitter, interacting with distinct subclasses of receptors referred to as quisqualate, amino-hydroxymethylisoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors (Watkins et al., 1990). NMDA receptor is a ligand-gated ion channel (Wang and Johnson, 1991), which has been most extensively studied and characterized in biochemical and behavioural experiments. Both competitive and noncompetitive antagonists (e.g. 2-amino-5-phosphonopentanoate (AP5) and MK-801, respectively) have been described for this receptor type. There is also evidence suggesting a role of excitatory amino acid transmission outside the central nervous system (CNS). Several binding studies have demonstrated that excitatory amino acid receptors are widely distributed, but not ubiquitous, in the mammalian peripheral tissues (Erdö, 1991).

MK-801 (dizocilpine: 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) blocks the NMDA-associated ion channel (Lodge and Johnson, 1990). This compound is an anticonvulsant agent that also possesses central sympathomimetic (Clineschmidt et al., 1982a, b) and psychotomimetic properties (Rogawski and Porter, 1990). Additionally, MK-801 has been found to be an inhibitor of NMDA-induced noradrenaline release and of catecholamine uptake in rat striatal synaptosomes (Snell et al., 1988). These effects are similar to those of phencyclidine (PCP), which binds to the same site as MK-801 in the NMDA receptor complex (Loo et al., 1987). It has been reported that TCP (1-[1-(2-thienyl) cyclohexyl]piperidine), a PCP receptor-specific ligand, potentiates the electrically evoked noradrenaline release in mouse vas deferens (Campbell et al., 1987) and preliminary data from our laboratory showed a possible action of MK-801 on the noradrenaline effect in isolated rat vas deferens. The aim of the present work was to study the effect of MK-801 on peripheral noradrenergic neurotransmission in rat vas deferens.

2. Materials and methods

After decapitation, vasa deferentia were removed from male Sprague-Dawley rats (200–250 g; CERJ, France), bathed with Krebs-Henseleit solution (composition in mM: NaCl, 119, KCl, 4.6, $CaCl_2$, 2.5, $NaHCO_3$, 25, KH_2PO_4,

* Corresponding author. Tel.: (34) (3) 4034531; fax: (34) (3) 4021886.

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1.2, $MgSO_4 \cdot 7H_20$, 1.2 and glucose, 11.1), and gassed with 95% O_2/5% CO_2. In experiments performed with low Na^+ concentration, NaCl was replaced by an equimolar amount of choline chloride. Tissues were freed of connective tissue and adhering fat, and bisected into the epididymal and the prostatic end.

For the organ bath experiments, the epididymal portions were mounted in 20-ml tissue chambers and attached to isometric strain-gauge transducers for recording. The organs were suspended under a resting tension of 400 mg at 37°C and allowed to stabilize for 60 min. Tension was amplified, recorded and sent to a computerized system running PROTO5 software (Letica Software Department, BCN, Spain) via a Letica LE 60–100 interface.

Reserpinized animals received a single dose of reserpine (5 mg/kg s.c.) 16 h before being killed.

Pharmacological agents used were: noradrenaline bitartrate, reserpine, prazosin HCl, yohimbine HCl, desipramine HCl, β-estradiol 3,17 disulfate, *N*-methyl-D-aspartate, glycine and choline HCl from Sigma (St. Louis, MO); (+)-MK-801 from Research Biochemical International (Natick, MA) and *levo*-[7-^{3}H]noradrenaline (specific activity 13.1 Ci/mmol) from New England Nuclear (Dreieich, Germany).

2.1. Electrically induced responses

Vasa deferentia were mounted between platinum field electrodes. After the equilibration period, contractions were elicited, for 10 min, by single pulses (0.1 Hz, 0.5 ms, Grass S88 stimulator) at supramaximal voltage.

2.2. Noradrenaline-induced contractile responses

Cumulative concentration-response curves for exogenous noradrenaline were performed in the absence (control curves) and in the presence of different concentrations of MK-801 ranging from 0.3 to 5.3 μM. MK-801 was added 10 min before starting the curves.

2.3. Experiments performed in low-sodium medium

In order to evaluate the influence of Na^+ concentration on the effect of MK-801, one vas deferens of each animal was bathed with Krebs-Henseleit solution (control) whereas the other was bathed with the low-sodium medium (25 mM). The responses were elicited by the addition of a single dose of noradrenaline (1.6 μM) in the absence and in the presence of MK-801 (5.3 μM).

2.4. Data analysis

EC_{50} values were calculated by PROTO5 software. All results are expressed as mean ± S.E.M. from n different experiments. A P value of < 0.05 was considered as significant. Differences between values were estimated using ANOVA followed by Scheffé's test.

The inhibition of noradrenaline uptake in organ bath experiments was quantified following the calculations proposed by Kenakin (1987). In brief, assuming that the uptake process follows Michaelis-Menten kinetics, the ratio [A]/[A'] refers to the organ-bath concentrations that produce a given response in the absence ([A]) and presence ([A']) of uptake inhibitor. K_I, defined as the equilibrium dissociation constant of the inhibitor-removal-site complex, can be calculated by the following equation:

$$\log\left[\frac{y(x-1)}{y-x}\right] = \log[I] - \log K_I$$

Where [I] is the inhibitor concentration; x quantifies the tissue sensitization to the agonist (i.e. the potentiation of response, [A/A'], produced by [I]) and y denotes the maximal sensitization obtained.

2.5. [^{3}H]Noradrenaline uptake

To evaluate the effect of MK-801 on noradrenaline uptake, the method described by Finberg et al. (1992) was followed with some modifications. Briefly, vasa deferentia were obtained as described above, cleaned and bisected transversally. Slices of the epididymal portions were gently blotted, weighed and placed in glass vials containing 10 ml of Krebs-Henseleit solution (with ascorbic acid 0.1 mM) gassed with 95% O_2/5% CO_2. The tissues were preincubated for 25 min at 37°C (or at 0°C and 3 mM noradrenaline when measuring the nonspecific incorporation). After the preincubation time, the slices were transferred to plastic tubes containing 0.95 ml of warmed and gassed Krebs-Henseleit solution. For the inhibition studies, the tissues were kept for 10 min in 0.95 ml of medium containing MK-801 at different concentrations. The incubation started by the addition of 50 μl of a solution of [^{3}H]noradrenaline to make a final concentration of 0.1 μM.

Incubation was carried on for a further 3 min, the tissues rapidly removed, rinsed in about 200 ml of ice-cold Krebs-Henseleit solution and transferred to scintillation vials containing 0.5 ml Solvable (DuPont, Germany). After 3 h solubilisation at 50°C, the vials were allowed to reach room temperature and 10 ml of Ecolite (+) cocktail (ICN Biomedicals) was added. Tritium content was estimated by liquid scintillation using a Beckman LS 1800 counter. The efficiency of counting was 40%. The total uptake was determined by correction, subtracting the 0°C uptake from that obtained at 37°C. The percentage of uptake in the presence of different concentrations of MK-801 was calculated in relation to the total uptake in the absence of this drug.

IC_{50} value was calculated by nonliner regression analysis using GraphPAD InPlot software (GraphPAD Software, San Diego, CA).

3. Results

3.1. Effect of MK-801 on electrically induced responses

MK-801 (3.7 μM) alone significantly increased the electrically induced responses (46.60% ± 5.09, $n = 6$, $P < 0.05$) (Fig. 1A) and the basal tone in a Mg-free Krebs-Henseleit solution. This effect was potentiated significantly by the presence of magnesium in the medium (251.75% ± 13.35, $n = 6$, $P < 0.002$) and, therefore, further experiments were performed with the Krebs-Henseleit solution described above.

3.2. Effect of MK-801 on resting tension

MK-801, at a concentration of 5.3 μM, induced spontaneous contractile responses, ranging from 0.15 to 0.95 g of tension, in the epididymal portion of rat vas deferens that remained for at least 30 min in the presence of MK-801 (Fig. 1C). This effect was completely abolished by prazosin (1 μM). Furthermore, these contractile responses did not appear in organs removed from reserpinized animals, although they did appear after 3 min of preincubation of the reserpinized tissues with 16 μM noradrenaline.

NMDA (300 μM) and glycine (10 μM) did not reverse these contractile responses.

3.3. Effect of MK-801 on noradrenaline-induced responses

MK-801 potentiated the effect of noradrenaline (Fig. 1B) in the epididymal portion of the rat vas deferens with a concentration-dependent reduction of the EC_{50} value for noradrenaline (see Table 1 and Fig. 2). This effect occurs in vasa deferentia from both control and reserpinized rats. An apparent K_I value of 1.53×10^{-9} M for MK-801 potentiation of noradrenaline responses was obtained.

Whereas the presence of yohimbine (0.1 μM) in the medium did not affect the potentiation of noradrenaline effect by MK-801 at the highest concentration tested (5.3 μM), equimolar substitution of NaCl by choline in the Krebs-Henseleit solution abolished this effect.

The potentiation of the concentration-response curves by MK-801 (5.3 μM) was not significantly modified by the previous addition of NMDA (300 μM) and glycine (10 μM): EC_{50} for noradrenaline = 0.14 ± 0.03 μM, $n = 7$, $P > 0.05$.

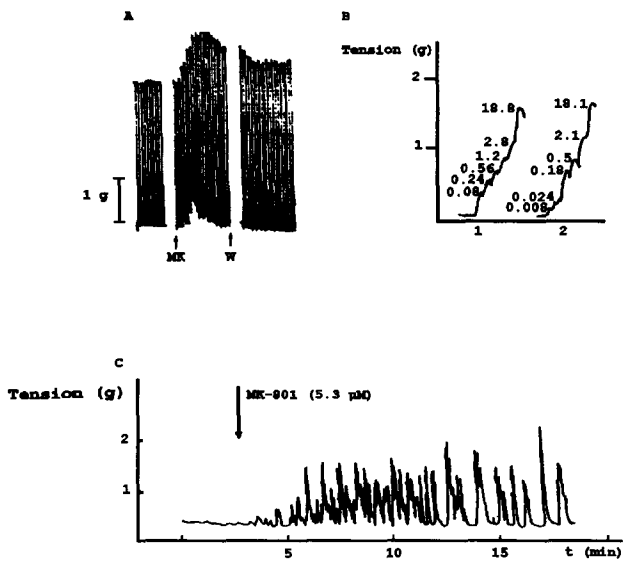

Fig. 1. A: Effect of MK-801 (3.7 μM; MK) on electrically induced contractions of the epidydimal portion of rat vas deferens; W indicates washout. B: Cumulative NA-induced responses in the absence (1) and in the presence (2) of MK-801 (0.53 μM); NA concentrations are indicated (μM) beside each curve. C: spontaneous contractile responses induced by MK-801 (5.3 μM) in rat vas deferens. Arrow indicates when the drug was added.

Table 1
Potentiation of NA concentration-response curves by MK-801

MK-801 (μM)	EC_{50} of NA (μM)	n
–	0.99 ± 0.11	14
0.3	0.57 ± 0.29 [a]	14
0.5	0.38 ± 0.02 [b]	19
1	0.23 ± 0.06 [b]	18
2.7	0.09 ± 0.009 [b]	12
5.3	0.06 ± 0.01 [b]	12

Data are expressed as mean ± S.E.M. from n different experiments. ANOVA and Scheffé's test: [a] $P < 0.01$, [b] $P < 0.001$ vs. control group (absence of MK-801).

3.4. [^{3}H]Noradrenaline uptake

MK-801 inhibited the [^{3}H]noradrenaline uptake into the epididymal portion of rat vas deferens in a range of micromolar concentrations, with an IC_{50} value of 1.79 ± 0.06 μM (Fig. 3). At 3 min incubation time, there was found no difference between the uptake in the absence and in the presence of 10 μM β-estradiol (in order to block

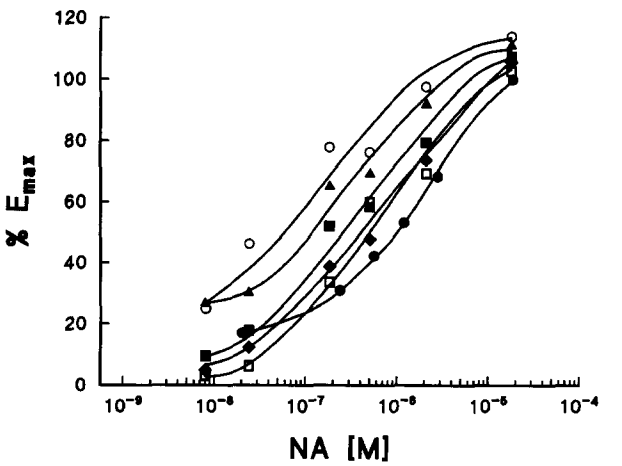

Fig. 2. NA concentration-response curves in the presence of different concentrations of MK-801. ● control, □ MK-801 0.3 μM, ◆ MK-801 0.5 μM, ■ MK-801 1 μM, ▲ MK-801 2.7 μM and ○ MK-801 5.3 μM.

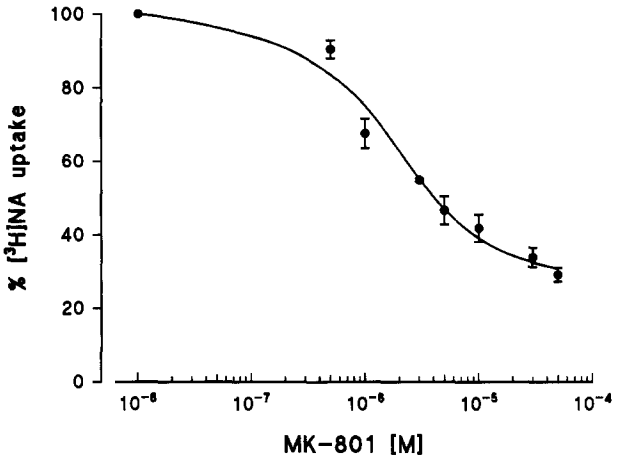

Fig. 3. Effect of MK-801 on the uptake of [^{3}H]NA into the epididymal portion of rat vas deferens. Tissues were incubated at 37°C for 3 min in the presence of [^{3}H]NA (0.1 μM) and increasing concentrations of MK-801. Each point represents mean uptake of three or more sets of duplicates; S.E.M. shown by vertical bars.

the nonneuronal uptake) and, therefore, further experiments were performed in the absence of this drug.

4. Discussion

The present study demonstrates that MK-801 can enhance the contractile responses to noradrenaline in the epididymal portion of rat vas deferens. This potentiation appears in electrically evoked contractions and also when exogenous noradrenaline is added to the organ bath. On the other hand, MK-801 alone induced spontaneous contractile responses that did not appear in organs originating from reserpinized rats (thus ruling out direct α_1 postsynaptic activation) but that could be achieved when the vasa deferentia were preincubated with exogenous noradrenaline, in order to recharge their endogenous stores. Moreover, a possible action on presynaptic α_2-adrenoceptor is ruled out by the finding that yohimbine did not modify the responses to MK-801.

To explain such effects, two mechanisms could be postulated: (1) MK-801 induces the release of endogenous noradrenaline; or (2) MK-801 blocks the noradrenaline uptake. The fact that the potentiation due to exogenous noradrenaline-induced contractions was still present in organs with depleted stores of cathecolamines (reserpinized rats) and that they did not show spontaneous contractile responses after the addition of MK-801, supports the hypothesis of the uptake inhibition induced by MK-801.

Most neurotransmitter transporters are Na^+ ion-dependent (Zeitner and Graefe, 1986; Worrall and Williams, 1994) and, therefore, a low concentration or the suppression of this ion in the medium reduces their activity. In our experiments, the replacement of NaCl by choline chloride in the Krebs-Henseleit solution prevented the potentiation of noradrenaline by MK-801. Although Na^+ was not totally removed (there was still 25 mM as $NaHCO_3$), the important reduction of its concentration and the presence of choline, that has been found to be a substrate of the neuronal noradrenaline carrier in the rat vas deferens (Ungell et al., 1986), allowed to reach an inhibition of the uptake system enough to establish comparisons with control tissues (bathed with standard medium). These results suggested a mechanism related with the uptake activity. In fact, desipramine alone, a specific inhibitor of neuronal cathecolamine uptake, elicited (0.1 μM) spontaneous contractile responses like those elicited by MK-801 (data not shown).

The potentiation of the exogenous noradrenaline effect by MK-801 occurred in the presence of magnesium as did the enhancement of the electrically evoked contractions. On the other hand, NMDA and glycine did not reverse this potentiation. These findings suggest that this action could not be linked to the ion-gated channel in the NMDA receptor complex.

PCP and σ receptor ligands have been reported to inhibit noradrenaline uptake in brain synaptosomes and adrenal chromaffin cells (Rogers and Lemaire, 1991) and also to inhibit, in a noncompetitive manner and at micromolar concentrations, the [^{3}H]desipramine binding in bovine adrenal medulla (Rogers and Lemaire, 1992). This effect is attributed by these authors to an interaction with an allosteric site on the noradrenaline transporter and not to the PCP or σ binding sites. Therefore, MK-801, as a PCP-site ligand, could block noradrenaline uptake in a similar manner.

In order to evaluate the effect of MK-801 on noradrenaline uptake, we measured the influence of MK-801 on the accumulation of [^{3}H]noradrenaline into slices of rat vas deferens. In our study, a 3-min period of uptake was used for measuring initial rate of uptake (Finberg et al., 1992). The results showed an inhibition of the noradrenaline accumulation by MK-801 that confirms the hypothesis suggested above.

Finally, the present work demonstrates the enhancement of the noradrenaline responses by MK-801 on a smooth muscle such as the rat vas deferens. This effect has been measured as a potentiation of the contractile responses on isolated organ and as an inhibition of the [^{3}H]noradrenaline uptake. Data obtained from these experiments indicate that these actions are unrelated to the ion-gated channel in the NMDA receptor complex and support the possible interaction of this drug with an allosteric site in the noradrenaline transporter as it has been suggested by some authors.

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