Cardiovascular Effects of the N-Methyl-D-Aspartate Receptor Antagonist MK-801 in Conscious Rats

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Evidence from microinjection studies in anesthetized rats suggests that central excitatory amino acid pathways using N-methyl-D-aspartate receptors are involved in the regulation of the cardiovascular system. To test the hypothesis that these pathways are tonically involved in the maintenance of or the baroreceptor reflex regulation of cardiovascular function, we have examined the effects of intravenous injection of the centrally acting, noncompetitive N-methyl-D-aspartate receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), on the mean arterial pressure, heart rate, renal sympathetic nerve activity, and behavior of conscious, freely moving sham-operated and sinoaortic baroreceptor-denervated rats. Administration of MK-801 produced, within 5 minutes, dose-dependent elevations in mean arterial pressure, heart rate, and renal sympathetic nerve activity that were sustained for 0.5 to 2.5 hours. For an equivalent dose, MK-801 produced approximately twice the peak changes in mean arterial pressure and heart rate in the sinoaortic baroreceptor-denervated rats than in the sham-operated rats. Pretreatment results were as follows: 1) The ganglion blocker chlorisondamine markedly attenuated the hypertension and tachycardia in the sham-operated and sinoaortic baroreceptor-denervated rats, 2) pretreatment with the α1-adrenergic receptor antagonist prazosin virtually abolished the hypertension, and 3) the β2-adrenergic receptor antagonist atenolol markedly reduced the tachycardia. MK-801 also produced stereotypic behaviors and ataxia in the sham-operated and sinoaortic baroreceptor-denervated rats; however, qualitatively and quantitatively similar changes in behavior were induced in the latter by doses approximately five times lower than required in the sham-operated rats. We conclude that 1) the MK-801-induced hypertension and tachycardia appear to result largely from centrally mediated sympathoexcitatory actions rather than only by inhibition of the baroreceptor reflex; 2) the cardiovascular changes produced by MK-801 may, in part, result from the behavioral excitation; and 3) tonically active excitatory amino acid pathways using N-methyl-D-aspartate receptors appear to be involved in the regulation of autonomic function. (Hypertension 1989;13:759-765)

There is now considerable evidence that neurons within the central nervous system and primary afferents terminating within the brainstem or spinal cord use excitatory amino acids (EAAs) such as L-glutamate or L-aspartate as neurotransmitters. These EAAs can interact with at least three subtypes of glutamate receptors designated as kainate, N-methyl-D-aspartate (NMDA), and quisqualate. Subpopulations of these EAA neurotransmitter pathways appear to be involved in the regulation of the cardiovascular system. For example, Gordon demonstrated that microinjection of D-2-amino-5-phosphonovaleric acid (D-AP5), a competitive antagonist of NMDA receptors, into the caudal ventrolateral medulla of rats increased arterial pressure and attenuated reflex-mediated decreases in mean arterial pressure (MAP) elicited by stimulation of the aortic depressor nerve. Guy-
enet et al\(^1\) reported that the microinjection of kynurenic acid, a glutamate receptor antagonist, into the nucleus tractus solitarii (NTS) or ventrolateral medulla attenuated the baroreceptor reflex-mediated changes in vagal and sympathetic outflow and raised arterial pressure. These studies raise the possibility that these (or other) EAA pathways are involved in the tonic regulation of autonomic function.

The present study describes several experiments designed to test the hypothesis that EAsas putatively involved in the regulation of the cardiovascular system, and especially those using NMDA receptors, are tonically active in conscious rats. In these studies, the novel compound MK-801\(^*\),\(^9\) a use-dependent noncompetitive antagonist of the NMDA subtype of glutamate receptors\(^10\) that readily enters the central nervous system on systemic injection,\(^9\) was used to assess the involvement of central NMDA receptors in cardiovascular function. Electrophysiological and radioligand-binding studies have clearly demonstrated that MK-801 specifically binds within ion channels associated with the NMDA receptor complex and that it does not interact with the kainate or quisqualate subtypes of the glutamate receptor (see Reference 10). We used 21 sham-operated and 21 sinoaortic baroreceptor–denervated (SAD) rats to determine whether the cardiovascular effects of MK-801 involve interactions with the baroreceptor reflex.

Materials and Methods

General Details

All experiments were performed on conscious, freely moving male Sprague-Dawley rats (Bio-Lab Corp., St. Paul, Minnesota) weighing 312±8 g. These rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (120 mg/kg) and acepromazine maleate (12 mg/kg) and subjected to either sham operation or sinoaortic baroreceptor denervation as described by Krieger.\(^11\) Twelve to 14 days after sham operations, rats were reanesthetized with the ketamine-acepromazine mixture, and polyethylene cannulas (PE-10 connected to PE-50, Clay Adams, Parsippany, New Jersey) were inserted first into the femoral artery for the subsequent measurement of arterial blood pressure. The rats were given 4–6 hours to recover before the experiments were started except for the study in which renal sympathetic nerve activity (RSNA) was recorded (see below).

To directly record the arterial blood pressure, the femoral arterial cannulas were connected to a pressure transducer (Century CP-01, Inglewood, California) linked to a Beckman recorder (model R611, Fullerton, California). The heart rate (HR) was derived from the arterial pulse pressure with a Beckman cardiotachometer (model 9857B).

Dose-Response Study

The magnitude and time course of the effects of selected doses of MK-801 on the MAP and HR of sham-operated (n=8) and SAD (n=8) rats were investigated. Sham-operated rats received 50–250 \(\mu\)g/kg doses of MK-801, whereas the SAD rats received 25–100 \(\mu\)g/kg doses. All rats received one dose per day over consecutive days, in a modified Latin-square dosage schedule.

Pharmacological Blockade

Sham-operated (n=8) and SAD (n=8) rats were pretreated with an intravenous bolus injection of either saline (0.9% wt/vol NaCl), the ganglion blocking agent chlorisondamine (2.5 mg/kg), the \(\alpha\)-adrenergic receptor antagonist prazosin (200 \(\mu\)g/kg), or the \(\beta\)-adrenergic receptor antagonist atenolol (1.0 mg/kg) 15 minutes before the injection of MK-801. The sham-operated rats received a 200-\(\mu\)g/kg dose of MK-801, whereas the SAD rats received 100 \(\mu\)g/kg. All rats received each of these pretreatments with the relevant dose of MK-801 over 4 consecutive days in a modified Latin-square design.

Renal Sympathetic Nerve Activity

Fourteen to 16 days after sham or SAD procedures, the rats received femoral arterial and venous cannulas as described above. Effenter renal sympathetic nerve activity was recorded by using the technique described by Thoren\(^12\) with minor modifications. In brief, the left renal artery and associated nerves were exposed via a flank incision. The left renal nerve was isolated and carefully freed from the surrounding tissue with the aid of a stereoscopic dissecting microscope. A thin platinum-iridium bipolar electrode was placed around the nerve and insulated with silicone rubber (Wacker Silicones, Adrian, Michigan). The electrode cable was sutured to the surrounding muscle, and the free end was tunneled subcutaneously to the back of the neck, exteriorized, and sutured in place. The rats were given 4–6 hours to recover from the anesthesia before commencement of the experiment. The nerve signals were amplified (×10,000) by a Grass P511 amplifier (Grass Instr. Co., Quincy, Massachusetts) with a low- and high-frequency cutoff set at 100 and 1,000 Hz, respectively. The amplified nerve activity was displayed on a Tektronix oscilloscope and was also channeled through a nerve traffic analyzer (Bioengineering Service, University of Iowa, Iowa City, Iowa) that measured the frequency of spikes exceeding the noise level (background, as determined by injection of the ganglion blocking agent trimethaphan, 10 mg/kg i.v.). The output from the nerve traffic analyzer and the MAP and HR were displayed on a Beckman Dynograph recorder (Beckman Instruments).

Statistics

Differences between group medians were analyzed by either Wilcoxon rank sum test, Kruskall-
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Wallis one-way nonparametric analysis of variance (ANOVA), or Friedman's two-way nonparametric ANOVA (for repeated-measures data), followed where necessary by the appropriate critical range tests for multiple comparisons of differences between medians. The differences between the slopes of the MAP or HR relations were analyzed by non-parametric orthogonal partitioning techniques described by Meddis. In all analyses, \( p < 0.05 \) was considered significant.

**Results**

**Dose–Response Effects of MK-801**

Peak changes in MAP and HR in sham-operated and SAD rats after the injection of selected doses of MK-801 are shown in Figure 1. Resting MAP and HR values before the injection of the four doses of MK-801, which averaged 107±3 mm Hg and 361±9 beats/min, respectively, were not significantly different from one another. In SAD rats, the MAP and HR values before MK-801 administration averaged 111±3 mm Hg and 363±12 beats/min, respectively, and also were not different from each other.

MK-801 produced dose-dependent increases in MAP and HR in both the sham-operated and SAD rats. All doses examined produced a significant hypertension and tachycardia \( (p < 0.05 \) for all comparisons). For an equivalent dose, MK-801 produced approximately twice the increase in MAP and HR in the SAD rats than in the sham-operated rats. The slope of the MAP dose–response curve (over the 25–100 \( \mu \)g/kg range) in the SAD rats was approximately twice that in sham-operated rats \( (5.1 \pm 0.6 \) vs. \( 2.2 \pm 0.4, p < 0.05 \)), and the slope of the HR dose–response curve in the SAD rats was approximately three times that in sham-operated rats \( (11.3 \pm 1.2 \) vs. \( 3.8 \pm 0.4, p < 0.05 \)).

**Time Course**

The time courses of the MK-801–induced changes in MAP and HR in the sham-operated and SAD rats are shown in Figure 2. In both groups, the peak
rises in MAP and HR occurred between 5 and 10 minutes after injection of MK-801. The duration of the hypertension and tachycardia was dose dependent in the sham-operated and SAD rats. For equivalent MK-801-induced rises in MAP and HR, the duration of the responses in the sham-operated and SAD rats were generally of equivalent length.

**Renal Nerve Activity**

Examples of the effects of MK-801 on MAP, HR, and RSNA of a sham-operated and SAD rat are shown in Figure 3. In both rats, MK-801 (200 and 100 μg/kg, respectively) increased MAP and HR within 5–10 minutes of injection. These changes were associated with temporally related increases in RSNA. A summary of the percentage changes in MAP, HR, and RSNA produced by MK-801 in the sham-operated and SAD groups is shown in Figure 4. In these experiments the 200-μg/kg dose of MK-801 in the sham-operated rats produced equivalent elevations in MAP and HR but slightly greater increases in RSNA than produced by the 100-μg/kg dose in the SAD rats.

**Mechanisms of Hypertension and Tachycardia**

The resting MAP and HR values 15 minutes after treatment (i.e., immediately before the injection of MK-801) with either saline, prazosin, atenolol, or chlorisondamine in the sham-operated and SAD rats are summarized in Table 1. The effects of pretreatment with these agents on the peak changes in MAP and HR produced by MK-801 in the sham-
operated (200 μg/kg dose) and SAD (100 μg/kg) rats are summarized in Figure 5. Gallion blockade markedly attenuated the MK-801-induced hypertension and tachycardia in both the sham-operated and SAD rats. Pretreatment with prazosin markedly reduced the hypertension but not the tachycardia, whereas atenolol virtually abolished the tachycardia without affecting the hypertension.

Behavioral Responses

The principle MK-801-induced behaviors observed with the lowest doses of MK-801 in the sham-operated (50 μg/kg) and SAD (25 μg/kg) rats were head nodding and weaving and later mild ataxia and dissociation or lack of response to environment changes (e.g., no exploration or other signs of recognition of being placed outside the home cage). With the higher doses (sham-operated rats, 100–250 μg/kg; SAD rats, 50–100 μg/kg), all rats displayed the head nodding and weaving; the majority of rats began to circle continuously (within 5–10 minutes of injection), and all rats developed marked ataxia and dissociation. Qualitatively similar changes in behavior could be induced in the SAD rats with lower doses than those required in the sham-operated rats. For example, the 50-μg/kg dose of MK-801 produced in the SAD rats behavioral changes qualitatively similar in magnitude to those produced by the 250-μg/kg dose in the sham-operated rats.

Discussion

The present study provides evidence that tonically active EAA pathways using NMDA receptors are involved in the central regulation of the cardiovascular system. This study demonstrates that the intravenous injection of the centrally acting, non-competitive NMDA receptor antagonist MK-801 produces sustained elevations in MAP and HR that are paralleled by increases in RSNA in conscious, freely moving sham-operated rats. Moreover, MK-801 produced qualitatively similar changes in SAD rats, although for an equivalent dose the hypertension, tachycardia, and increase in RSNA were approximately twice that observed in the sham-operated group. In terms of the mechanisms by which MK-801 produces its cardiovascular actions, it was found that pretreatment with 1) the ganglion blocker chlorisondamine markedly reduced the MK-801-induced hypertension and tachycardia in both the sham-operated and SAD rats, 2) the α1-adrenergic receptor antagonist prazosin markedly reduced the hypertension but not the tachycardia, and 3) the β1-adrenergic receptor antagonist atenolol virtually abolishes the tachycardia without affecting the hypertension.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial +15 min</td>
<td>Initial +15 min</td>
<td>Initial +15 min</td>
<td>Initial +15 min</td>
</tr>
<tr>
<td>Saline</td>
<td>106±3</td>
<td>105±2</td>
<td>348±12</td>
<td>344±14</td>
</tr>
<tr>
<td>Prazosin</td>
<td>105±2</td>
<td>97±2*</td>
<td>355±9</td>
<td>361±11</td>
</tr>
<tr>
<td>Atenolol</td>
<td>103±3</td>
<td>102±2</td>
<td>362±8</td>
<td>330±9*</td>
</tr>
<tr>
<td>Chlorisondamine</td>
<td>105±1</td>
<td>56±2*</td>
<td>345±12</td>
<td>296±13*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=8 rats per group). +15 minute values represent those immediately before MK-801 injection. SAD, sinoaortic baroreceptor-denervated; MAP, mean arterial pressure; HR, heart rate.

*p<0.05, comparing +15 minutes with initial.
These findings suggest that the MK-801-induced hypertension and tachycardia arise from actions within the brain. Further evidence for the central action of MK-801 comes from preliminary unpublished findings that the administration of D-AP5 (1 mg/kg i.v.), an NMDA receptor antagonist that poorly penetrates the brain (see Reference 10), failed to alter arterial pressure, HR, or behavior. The results in rats with sinoaortic baroreceptor denervation suggest that the cardiovascular effects of MK-801 cannot be explained solely by inhibition of the baroreceptor reflex. Indeed, one interpretation of these findings is that the baroreceptor reflex may effectively limit the expression of the cardiovascular actions of MK-801. Since we did not determine the effects of MK-801 on the baroreceptor reflex per se, it is possible that MK-801-induced hypertension and tachycardia observed in rats with intact baroreceptor reflexes involve inhibition of this reflex as well as sympathoexcitation. In support of this suggestion, Gordon2 has reported that the disinhibition of the SAD rats were not investigated. Possible explanations that will require examination include 1) alteration of the activity of EAA pathways in the brain. Further evidence for the central action of MK-801 comes from preliminary unpublished findings that the microinjection of excitatory amino acid antagonists, including a specific NMDA receptor antagonist D-AP5, into the ventrolateral medulla produce a marked increase in arterial blood pressure. However, the hypertensive responses were accompanied by bradycardia, unlike the MK-801-induced hypertension that was associated with tachycardia.1 This suggests that, although the MK-801-induced hypertension may be due to actions within the ventrolateral medulla, the tachycardia probably results from noncompetitive inhibition of NMDA receptors elsewhere in the central nervous system. The possible sites for the cardiovascular actions of MK-801 (based on autoradiographic studies of the distribution of NMDA receptor sites)3-8 include the NTS, pons, and brainstem motor nuclei.

The present results are in agreement with a previous report that the hypertension and tachycardia produced by the dissociative anesthetic ketamine, also a noncompetitive NMDA receptor antagonist,10 are due to centrally mediated increases in sympathetic nerve activity (using plasma catecholamines as an index of sympathetic function).15 The effects of ketamine have also been attributed to 1) inhibition of the baroreceptor reflex,16 2) direct release of norepinephrine from sympathetic nerve terminals via a tyraminergic action,17 3) inhibition of reuptake of norepinephrine into sympathetic nerve terminals,18 and 4) β-adrenergic receptor-mediated increase in renin release.19 The present results did not determine to what extent mechanisms 2) to 4) contribute to the cardiovascular actions of MK-801. However, such actions would significantly augment the effects of increased sympathetic nerve activity. The stereotypic behaviors (e.g., circling and head weaving) and ataxia produced by MK-801 are consistent with a similar pattern of behavior produced by phencyclidine,20 which is also a noncompetitive antagonist of NMDA receptor sites.10 On the basis of the behavioral response study it was found that the SAD rats were approximately five times more sensitive than the sham-operated ones with respect to the production of these behaviors. The mechanisms involved in the increased behavioral sensitivity of the SAD rats were not investigated. Possible explanations that will require examination include 1) an alteration of the activity of EAA pathways in

![Figure 5](http://hyper.ahajournals.org/)

**FIGURE 5.** Effects of pretreatment with saline (SAL), chlorisondamine (CLX), prazosin (PRZ), or atenolol (ATN) on peak increases in mean arterial pressure (MAP) and heart rate (HR) produced by 200 μg/kg MK-801 in sham-operated rats and 100 μg/kg in sinoaortic baroreceptor-denervated (SAD) rats. Each value represents the mean±SEM (n=8 rats per group) of the peak changes. *p<0.05, comparing MK-801-induced changes after CLX, PRZ, and ATN pretreatment versus saline-pretreatment values.
the central nervous system, 2) increased penetrability of MK-801 into the central nervous system, and 3) a generalized increase in the behavioral reactivity of the SAD that does not necessarily involve a disturbance of NMDA receptors or EAA function. Although the behaviors become evident several minutes after the arterial blood pressure and HR have risen to their plateau levels (i.e., within 5-10 minutes), the possibility that the MK-801-induced hypertension and tachycardia is behaviorally induced cannot be discounted.

In summary, the present results demonstrate that MK-801 produces hypertension, tachycardia, and stereotypic behaviors probably via the noncompetitive inhibition of NMDA receptors within the central nervous system. We conclude that EAA neurotransmitter pathways using NMDA receptors may play important roles in the regulation of cardiovascular and behavioral systems.

Acknowledgment

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References

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