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Cardiovascular Effects of NMDA and MK-801 Infusion at Area Postrema and mNTS in Rat

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TIAN, B. AND D. K. HARTLE. *Cardiovascular effects of NMDA and MK-801 infusion at area postrema and mNTS in rat.* PHARMACOL BIOCHEM BEHAV **49**(3) 489–495, 1994. – In urethane-anesthetized male Sprague-Dawley rats, microinfusion of *N*-methyl-D-aspartate (NMDA) into the area postrema (AP) at the dose of 10 ng produced significant decreases in mean arterial pressure (MAP) (−26 ± 5 mmHg), heart rate (HR) (−34 ± 6 bpm), renal blood flow, mesenteric blood flow, and iliac vascular resistance. In addition, microinfusion of the same dosage of NMDA into the medial nucleus tractus solitarius (mNTS) produced significant decreases in MAP (−33 ± 4 mmHg), HR (−33 ± 6 bpm), renal blood flow, mesenteric blood flow and vascular resistance, and iliac blood flow and resistance. MK-801 (dizocilpine) microinfusion alone produced no significant changes in MAP or HR when microinfused either into the AP or unilaterally into the mNTS; however, bilateral microinfusion of MK-801 into mNTS produced sustained hypertension and tachycardia, lasting about 30 min. MK-801 pretreatment at both AP and mNTS effectively blocked NMDA-induced cardiovascular responses. MK-801 microinfusion at AP significantly attenuated baroreceptor reflex-mediated bradycardia elicited by intravenous injection of phenylephrine, but did not alter reflex tachycardia elicited by intravenous nitroprusside. In conclusion, NMDA receptor-mediated neurotransmission is involved in the cardiovascular functions of both AP and mNTS. Both loci appear to be sites of action for MK-801.

N-Methyl-D-aspartate MK-801 Arterial blood pressure Heart rate Baroreflex Area postrema Nucleus tractus solitarius Dizocilpine Circumventricular organ

EXCITATORY amino acid (EAA) transmission is known to be involved in regulation of the cardiovascular system at multiple sites in the central nervous system (CNS), such as the nucleus tractus solitarius (NTS) (23), caudal ventrolateral medulla (31), rostral ventrolateral medulla (17), lateral parabrachial nucleus (4), and intermediolateral cell column of the spinal cord (14). The *N*-methyl-D-aspartic acid (NMDA) receptor subtype is one of the five subtypes of glutamate receptors known to exist in the CNS, and appears to be involved at many of its sites (7).

The noncompetitive selective NMDA antagonist, dizocilpine, [(+)-5-methyl-10,11-dihydro-5H-dibenzo-(a,d) cyclohepten-5,10-imine maleate], or MK-801, is a useful tool for assessing the role of NMDA receptor-mediated excitatory amino acid neurotransmission in select brain loci. Compounds in this category are currently being investigated and developed as potentially useful agents in managing the neuroexcitotoxic sequelae attending various CNS insults, such as stroke (5), hypoxic brain ischemia (24), and seizure (34). NMDA antagonists have experimentally been proven to be effective in limiting excitatory amino acid mediated neurotoxicity (3).

Because NMDA-type EAA neurotransmission is used in so many cardiovascular regulatory regions of the CNS, it is not surprising that peripheral administration of MK-801 has been shown to produce significant cardiovascular effects mediated via the central nervous system (21). Peripheral administration of MK-801 in conscious rats produces hypertension and tachycardia that are accentuated by sinoaortic-deafferentation, but attenuated by ganglionic blockade (21). Therefore, both the blood pressure and the heart-rate effects appear to be mediated via the CNS and partially buffered by the arterial baroreflex. The focus of these studies was to test whether the area postrema (AP) and/or medial nucleus tractus solitarius (mNTS) sites of NMDA receptor-mediated excitatory amino acid transmission may participate in the cardiovascular effects of systemically administered MK-801 in rats. We predict that these side effects of MK-801 will be major considerations in the therapeutic use of NMDA antagonists as neuroprotectants.

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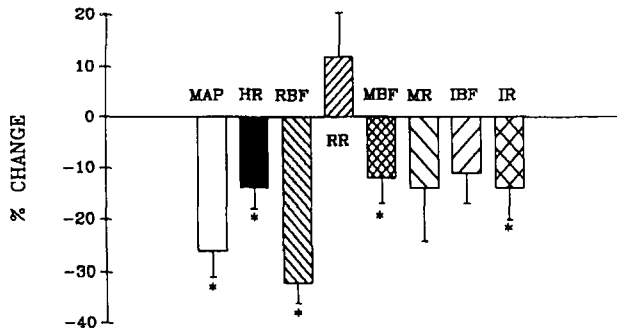

FIG. 1. Hemodynamic effects of infusion of NMDA (10 ng/10 nl per min for 1 min) into AP. $n = 8$, mean ± SEM. $*p < 0.05$ compared with baseline value.

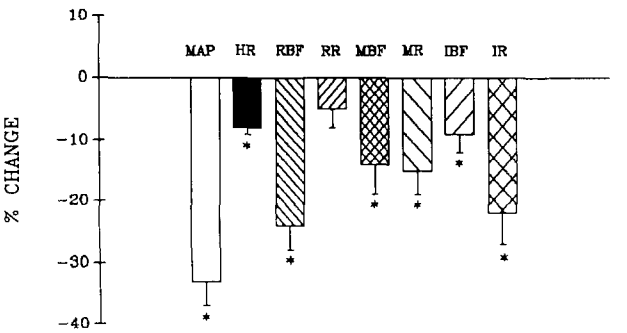

FIG. 2. Hemodynamic effects of unilateral infusion of NMDA (10 ng/10 nl per min for 1 min) into mNTS. $n = 8$, mean ± SEM. $*p < 0.05$ compared with baseline value.

We monitored blood pressure parameters, heart rate, and regional blood flows in renal, mesenteric, and iliac vascular beds during direct microinfusions of NMDA and MK-801 into the AP and during both unilateral and bilateral microinfusions into the mNTS. The effect of MK-801 microinfusion into AP on baroreflex-mediated heart-rate changes elicited by intravenous (IV) injection of either phenylephrine or nitroprusside was also tested.

METHODS

Animals and Housing Conditions

Male Sprague-Dawley (SD) rats (Charles River, Raleigh, NC) weighing 300–380 g were housed on a 12 h : 12 h light-dark cycle and allowed free access to standard laboratory Purina rat chow and tap water. All protocols were approved by the University of Georgia's Animal Care and Use Committee.

Surgical Preparations

The animals were anesthetized with urethane (1–1.2 g/kg, intraperitoneally). Left femoral artery and venous catheters (tapered PE-50 tubing) were then inserted for monitoring blood pressure and administration of drugs, respectively. Body temperature during anesthesia was maintained at 37.0 ± 1.0°C using an automatic electric heating pad. The arterial line was attached to a Statham small volume displacement pressure transducer, and blood pressure was continuously recorded on a Grass Model 79 C polygraph data recording system. HR was determined from the systolic peak-to-peak time intervals of the direct arterial blood pressure signal.

Next, a midline laparotomy was made and 5-mm lengths of the superior mesenteric, the left renal, and the right iliac arteries were exposed using a dissecting microscope to avoid damage. Miniaturized pulsed-Doppler flow probes, embedded in silastic cuffs (Titronic Medical Instrument, Iowa City, IA), were filled with ultrasound transmission gel and secured in place on each artery. Regional blood flows were monitored with a directional pulsed-Doppler flowmeter (model 545C-4; University of Iowa, Bioengineering Facility, Iowa City, IA) and signals were recorded on the polygraph. Changes in regional vascular resistance were calculated from peak changes in MAP and concomitant changes in regional blood flow caused by drug microinfusion treatments at either AP or mNTS. The percent change in regional vascular resistance during a treatment was calculated by expressing the regional vascular resistance change during the treatment as a percent of the relative regional resistance before treatment.

Stereotaxic Brain Surgery and Drug Microinfusions

Each animal was placed in a stereotaxic frame with its head flexed anteriorly downward at an angle of 30° from the horizontal plane between lambda and bregma. A dorsal cervical midline incision was made for removal of atlanto-occipital membrane and exposure of the AP and dorsal medullary surface. The total time required for all preparative surgery (abdominal flow probe placements and stereotaxic brainstem surgery) was <45 min from induction of surgical anesthesia. Thirty minutes was allowed after the surgery for stabilization of hemodynamic parameters before initiation of experimental treatments. An average length experiment involved a total of 2.0–2.5 h of anesthesia. No anesthesia supplements were necessary within those time limitations. Each experiment (Figs. 1–8) was performed independently of the others. A total of 47 rats were used in these experiments. This number excludes rats that were used to test for infusion doses and rates in preliminary trials to design the experimental protocols used in these experiments.

Microinfusions of either vehicle solution or drugs into AP or mNTS were performed using glass micropipettes (30-μm tip diameter) attached to a nanopump (Model A 1400; World Precision Instruments). The micropipette was backfilled with

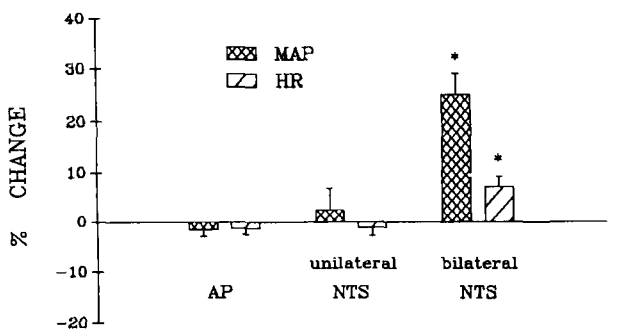

FIG. 3. Effects of infusion of MK-801 into AP, unilaterally into mNTS (20 nl/min for 5 min, 0.6 μg) or bilaterally into mNTS (20 nl/min for 3 min/side, 0.72 μg) on MAP and HR. $n = 8$, mean ± SEM. $*p < 0.05$ compared with baseline value.

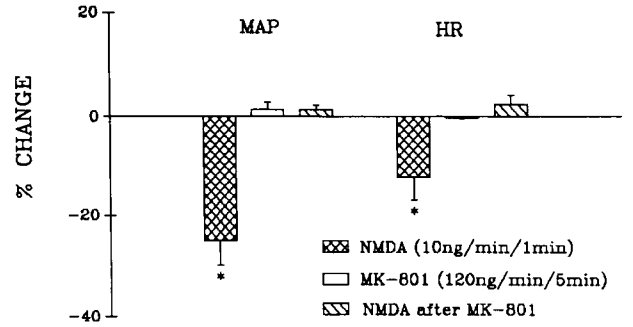

FIG. 4. Effects of MK-801 (20 nl/min for 5 min, 0.6 μg, AP) on the alteration of MAP and HR evoked by infusion of NMDA (10 ng/10 nl per min for 1 min) into AP. $n = 5$, mean ± SEM. $^*p < 0.05$ compared with baseline value.

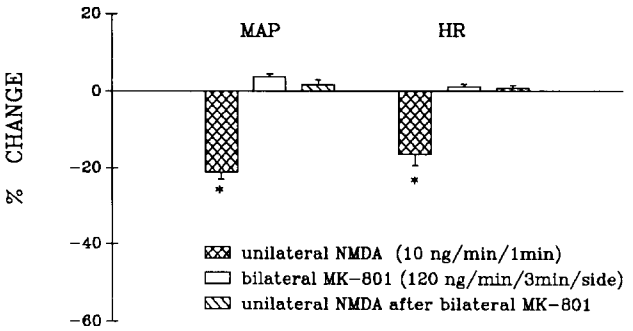

FIG. 6. Effects of bilateral administration of MK-801 (20 nl/min for 3 min/side, 0.72 μg) within mNTS on the alteration of MAP and HR evoked by infusion of NMDA (10 ng/10 nl per min for 1 min) into unilateral mNTS. $n = 7$, mean ± SEM. $^*p < 0.05$ compared with baseline value.

either a drug or a vehicle solution and positioned over the middle AP or the mNTS. The micropipette tip was then positioned on the midline and inserted 100 μm below the surface of the AP, midway between its rostral and caudal extent, at stereotaxic coordinates determined from the AP surface coordinates. Microinfusion into the mNTS was made by positioning the tip of the micropipette 500 μm lateral to the AP midline coordinates, but at a depth of 500 μm from the surface of the brainstem. When bilateral infusions were made into mNTS, the micropipette was immediately withdrawn after the microinfusion on one side and repositioned stereotaxically into the contralateral mNTS (elapsed time ~30 s).

Histology

At the end of experiment, the injection site was marked by similar microinfusion of an equivalent volume of concentrated solution of Evan's blue dye into either AP or mNTS sites. The anesthetized rat was then sacrificed, and its brain was removed and immediately frozen. The positions and diffusional extent of injections were mapped during light microscopic examination of serial 30-μm fresh frozen coronal sections across the AP region of the medulla. A composite drawing showing the maximal extent of the stained area was prepared using the subpostremal NTS atlas of Barraco et al. (2).

Source of Drugs

N-methyl-D-aspartate, phenylephrine, and nitroprusside were purchased from Sigma Chemical Co. MK-801 was purchased from Research Biochemical. The vehicle for all drug solutions was a sterile plasma electrolyte solution at pH 7.4 (Plasma-Lyte, Baxter Healthcare Corporation). The pH of all drug solutions was adjusted to 7.4 before microinfusion. The effect of isovolumic vehicle microinfusion was tested at the beginning of each experiment in both AP and mNTS sites. These tests were uniformly found to be without effect on any of the recorded variables.

Statistical Analyses

All data are presented as experimental group mean ± SEM. Percent changes in MAP, HR, regional blood flow, and regional vascular resistance were calculated from data obtained coincident with the peak changes in MAP that occurred relative to pretreatment values. Student's *t*-test was used to determine the statistical significance of the difference between single comparisons. One-way or two-way analysis of variance (ANOVA) procedures were used when appropriate to assess homogeneity of groups variances and to determine whether there were differences between group means. The ANOVA was followed by the least significant difference post hoc procedure for determination of statistical significance of

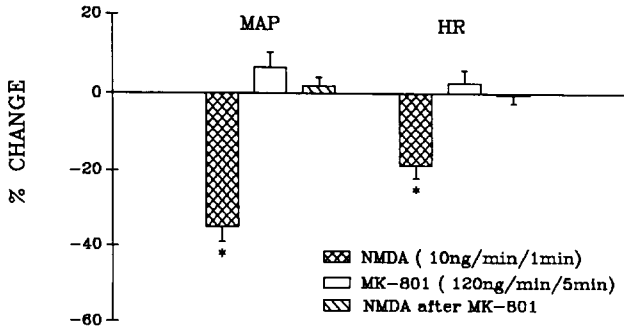

FIG. 5. Effects of MK-801 (20 nl/min for 5 min, 0.6 μg, unilateral mNTS) on the alteration of MAP and HR evoked by unilateral infusion of NMDA (10 ng/10 nl per min for 1 min) into mNTS. $n = 7$, mean SEM. $^*p < 0.05$ compared with baseline value.

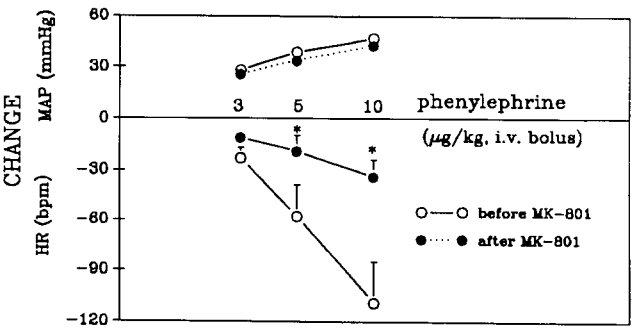

FIG. 7. Effects of MK-801 (20 nl/min for 5 min, 0.6 μg) infusion within AP on the phenylephrine. $n = 8$, mean ± SEM. $^*p < 0.05$ compared with groups.

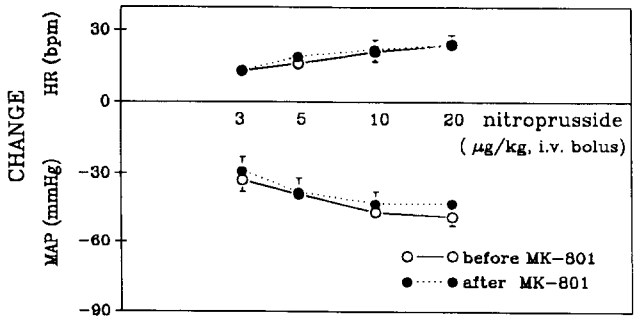

FIG. 8. Effects of MK-801 (20 nl/min for 5 min, 0.6 mg) infusion into AP on the changes of HR induced by IV administration of nitroprusside. n = 6, mean ± SEM.

differences. The criterion for significance was $p \leq 0.05$ in each comparison.

RESULTS

Cardiovascular Effects of NMDA or Vehicle Microinfusion in AP

Baseline MAP before microinfusion was 101 ± 5 mmHg, and baseline HR was 301 ± 45 beats/min (bpm). Microinfusion of NMDA (1 ng/nl) at a constant rate of 10 nl/min for 1 min in AP produced significant decreases in MAP (−26 ± 5 mmHg) and heart rate (HR) (−34 ± 6 bpm). These changes were accompanied by significant decreases in renal blood flow (−30%), mesenteric blood flow (−12%), and iliac vascular resistance (−14%) (Fig. 1). Arterial pressure began to decrease within 30 s from the onset of the microinfusion and lasted for 5–10 min after the microinfusion was discontinued. Renal vascular resistance, mesenteric vascular resistance, and iliac blood flow did not change significantly during NMDA treatment.

Cardiovascular Effects of NMDA Microinfusion in mNTS

Responses in MAP (−33 ± 4 mmHg) and HR (−30 ± 6 bpm) that occurred during microinfusion of NMDA (1 ng/nl) at a constant rate of 10 nl/min for 1 min (Fig. 2) unilaterally into mNTS were similar to those obtained in AP (Fig. 1). These changes were accompanied by significant decreases in renal blood flow (−24%), mesenteric blood flow (−14%), mesenteric vascular resistance (−15%), iliac blood flow (−9%), and iliac vascular resistance (−22%) (Fig. 2). There were no statistically significant differences between changes recorded during NMDA microinfusion in AP vs. mNTS in MAP, heart rate, regional blood flow, or calculated regional vascular resistance parameters. Vehicle microinfusion caused no significant changes in MAP or HR from baseline in either the AP or mNTS sites.

MK-801 Treatment of AP and mNTS

MK-801 (20 nl/min/5min, 6 ng/nl) microinfusion into AP or unilaterally into mNTS did not change MAP and HR. However, bilateral microinfusion of MK-801 (20 nl/min/3 min/side) into mNTS elicited significant increases in MAP (24 ± 2 mmHg) and HR (22 ± 4 bpm) (Fig. 3). The increases in MAP and HR began 30–60 s after initiation of the microinfusion at the contralateral mNTS injection site and lasted at least 15 min after that microinfusion period was terminated. No significant changes in iliac blood flow, iliac vascular resistance, renal blood flow, renal vascular resistance, mesenteric blood flow, or mesenteric vascular resistance accompanied these changes in MAP and HR (data not shown) during bilateral microinfusion of MK-801 at mNTS.

Blockade of NMDA With MK-801

Figures 4 and 5 presented data demonstrating that pretreatment of AP or unilateral mNTS with MK-801 (20 nl/min/5 min, 6 ng/nl) completely blocked the MAP and HR effects of NMDA (10 nl/min/1 min, 1 ng/nl) subsequently microinfused at the same site. We found that although the effect of the agonist lasted only 5–10 min, the antagonism of NMDA with MK-801 lasted about an hour. This was tested by periodic NMDA challenges after MK-801 (every 15 min) in a separate group of rats. We also found that when NMDA was unilaterally microinfused into mNTS before bilateral microinfusion of MK-801 into mNTS, the hypertensive and tachycardic effects observed during the treatment of the contralateral mNTS (see Fig. 3) were completely suppressed (Fig. 6).

Effect of MK-801 on Baroreflex Control of HR

The effect of MK-801 microinfusion in AP on the baroreflex control of HR during baroreceptor loading was tested with pressor challenges produced by IV administration of the α1-adrenergic receptor agonist, phenylephrine; similarly, baroreceptor unloading with the vasodilating agent, nitroprusside, was also tested. Phenylephrine injections increased blood pressure and decreased HR in a dose-dependent manner (Fig. 7). After MK-801 microinfusion into AP, bradycardic responses during PE challenges were significantly attenuated (Fig. 7), but MAP responses to phenylephrine were not altered. The duration of this apparent disengagement of the decelerator limb of the baroreflex by MK-801 was tested by periodic (every 10 min) phenylephrine pressor challenges. Attenuation of baroreflex-mediated bradycardia persisted for at least an hour after MK-801 microinfusion into AP. We also tested the effect of MK-801 microinfusion into AP on baroreflex-mediated cardioaccelerator responses elicited by nitroprusside injections. We found that although nitroprusside reproducibly caused dose-dependent decreases in MAP accompanied by increases in HR, pretreatment of AP with MK-801 had no significant effect on either HR or MAP responses to nitroprusside challenge (Fig. 8).

Light microscopic examination of the position of Evan's blue dye verified the correct position of the microinfusions in AP or mNTS. Figure 9 is a composite schematic representation of the areas flooded by dye during the AP and mNTS infusions. We observed that Evan's blue dye microinfused into mNTS did not diffuse into AP, into the commissural NTS, or to the contralateral mNTS. Laterally, blue dye diffused only as far as the tractus solitarius. No staining was observed in the lateral region of NTS. The blue-stained area included the mNTS, part of the dorsal strip subnucleus, dorsal to the mNTS, and the dorsalmost portion of the dorsal nucleus of the vagus. Evan's blue dye microinfused at AP did not diffuse into subjacent regions of NTS. The area shaded represents the approximate visual image of the Evan's blue dye. The area probably overestimates the effective drug treatment area produced by drug microinfusions because Evan's blue dye binds tightly to proteins and is not readily flushed from the brain. These histologic controls confirmed the proper placement of the tip of the micropipette for radial drug diffusion.

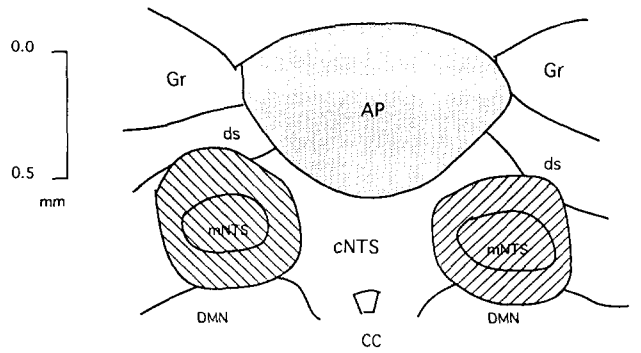

FIG. 9. Schematic drawing of the medial dorsal medulla of rat brainstem at the coronal level of the microinfusion into AP and mNTS (approx. −13.80 mm from Bregma). Stippled area shading AP indicates extent of Evan's blue dye staining after injection in middle AP. Cross-hatched area indicates average extent of dye staining after bilateral injection in mNTS. AP, area postrema; NTS, nucleus tractus solitarius; cNTS, commissural NTS; mNTS, medial NTS; ds, dorsal strip of NTS; Gr, gracile nucleus; DMV, dorsal motor nucleus of the vagus; cc, central canal.

DISCUSSION

MK-801 is a selective, noncompetitive, but use-dependent NMDA receptor antagonist designed to cross the blood-brain barrier after peripheral IV administration. Its lipid solubility allows it to diffuse into the brain in concentrations sufficiently to reduce NMDA receptor-mediated neuroexcitotoxicity (5,6,12). NMDA receptor antagonists are currently being developed for clinical management of stroke patients. Experimentally, they have proven useful in preventing secondary neuronal damage caused by excitotoxic episodes subsequent to the neural damage and neurochemical imbalances produced during the initial stroke insult (5). The strategy for the use of these agents is to achieve the therapeutic goal of dampening NMDA-mediated neuroexcitotoxicity without blocking vital NMDA receptor-mediated neurotransmission. The noncompetitive NMDA receptor-linked ion channel blockers have the advantages of being highly selective and use-dependent, and they cannot be competed off these sites by endogenously released agonists. These properties make them more useful clinically than the competitive antagonists for the NMDA receptor.

Lewis et al. (21) demonstrated that peripheral administration of MK-801 increases MAP, HR, and renal sympathetic nerve activity in the rat in a dose-dependent manner. In their study, responses obtained from 25 or 250 mg/kg MK-801 IV were sustained for 0.5 and 2.5 h, respectively. Because the blood pressure and heart-rate responses were effectively blocked by ganglionic blockade, they concluded that these MK-801 actions were produced via the CNS. They further suggested that possible sites for the cardiovascular actions of MK-801 included the NTS, pons, and brainstem motor nuclei. The data we obtained suggest that the AP and mNTS region are likely central sites of action contributing to the hypertensive and tachycardiac effects of MK-801.

The NTS is adjacent to the AP and is associated both anatomically and functionally with the AP in the regulation of cardiovascular system. Because the AP is relatively devoid of a blood-brain barrier, its neural elements are accessible to free plasma concentrations of many substances. The AP can therefore function as a chemosensory region. The AP is densely populated with receptors for many blood-borne substances that affect autonomic regulation, such as angiotensin II, vasopressin, endothelin, and atrial natriuretic peptides. The neuronal elements of AP are also richly endowed with receptors for many neurotransmitter and neuromodulator substances, including excitatory amino acid receptors (33).

After IV administration of MK-801, the AP is exposed to free plasma levels of the antagonist because of its highly dense and permeable capillary network specialized for high volume, but slow blood flow (10,11). The subjacent NTS would be among the first regions of the brain to attain high levels of MK-801. In support of this statement, the AP and underlying NTS are perfused by serially connected capillary beds, suggesting a rapid portal system to the NTS for lipid-soluble humoral agents such as MK-801 (28). The medial, caudal commissural NTS has capillary structures and permeabilities similar to AP (11) and may share the humoral chemosensory functions of the circumventricular organ. The mNTS and dorsal strip of the NTS have capillary structures, permeabilities, and blood-to-brain transfer constants for α-aminoisobutyric acid that are consistent with a more highly developed blood-brain barrier (11). AP neurons in turn project to several regions behind the blood-brain barrier that are known to be cardiovascular regulatory regions, such as the NTS, PBL, RVLM, dorsal motor nucleus of the vagus (DMN), and nucleus ambiguus (NA) (25). The AP and NTS each receive afferent information from arterial baroreceptors, cardiopulmonary receptors, arterial chemoreceptors (16), and descending afferents from higher cardiovascular regulatory centers in the CNS (32). The mNTS is also a major projection field for AP efferents (25), and may receive portal blood from the AP (28).

As mentioned previously, excitatory amino acid transmission is known to be involved in cardiovascular regulation mediated within the NTS. Glutamate has long been hypothesized to be an endogenous neurotransmitter in NTS cardiovascular circuits (15,30). The NTS has a highly excitatory amino acid content, a high-affinity excitatory amino acid uptake system, and dense NMDA receptors (26). Increased glutamate release has been measured in NTS during increased afferent vagal stimulation (9). Glutamate has been postulated by some to be one of the neurotransmitters of vagal primary afferents (27,30). Others suggest that glutamate is not the neurotransmitter of the primary baroreceptor afferents, but may be involved in secondary interneuronal transmission within cardiovascular integrative circuitry of the NTS (29). Evidence supporting the latter is that glossopharyngeal and vagal afferents appear to lack the high-affinity uptake and retrograde transport system for aspartate that characterizes neurons using glutamate or aspartate as neurotransmitters (29). Whereas excitatory amino acid transmission involved in NTS cardiovascular regulation and is required for aortic depressor nerve reflexes (19,21), one group proposes that non-NMDA receptor-mediated transmission may mediate the NTS relay of this specific reflex (8). Others, however, find that a significant portion of both the depressor and bradycardic effects of aortic nerve stimulation are blocked by either competitive (18) or noncompetitive (19) specific NMDA antagonism.

Although the function of specific synapses within NTS that use excitatory amino acid neurotransmission remains undetermined, it is widely accepted that excitatory amino acid neurotransmission within the NTS plays an important role in its cardiovascular regulation. Localized injections of NMDA into the NTS produces profound hypotension and bradycardia (8,18,19,30). Local microinjection of the competitive NMDA antagonist, D-AP5 (D-2-amino-5-phosphonovaleric acid) (8)

or the broad-spectrum excitatory amino acid antagonist, kynurenic acid (20), blocks the cardiovascular effects of locally injected NMDA and appears to block some reflex control pathways (8,20,35). Although the aortic depressor nerve reflex has been the most extensively studied, it should be remembered that phenylephrine-induced bradycardia is primarily mediated by carotid baroreceptors. Cardiovascular effects of direct microinjections of drugs could be simultaneously influencing baroreceptor functions, cardiopulmonary functions, and chemoreceptor functions of the NTS (35) and AP. The neurotransmitter substances involved in each of these pathways are also in dispute; however, excitatory amino acids are among the contenders. Because both the AP and mNTS are known to be involved in HR and MAP regulation, we chose to selectively treat these sites with NMDA and MK-801 using a direct microinfusion technique to test discretely the possible contributions of these regions to the cardiovascular responses observed after IV MK-801 administration (21).

The data obtained in this study demonstrated the hemodynamic consequences of NMDA and MK-801 at either AP or mNTS. Infusion of NMDA into either AP or unilaterally into mNTS produced similar depressor and bradycardic responses accompanied by similar changes in renal, mesenteric, and iliac blood flow. Decreases in mesenteric and iliac resistances did not reach statistical significance during AP microinfusion, but did in the mNTS microinfusion. Further comparisons between the AP and mNTS treatments revealed no statistically significant differences between the resistance responses in these vascular beds. Renal resistance did not change significantly during infusions at either site. In summary, the pattern of cardiovascular changes elicited by NMDA injection in either the AP or the mNTS was the same for all three vascular beds tested. This suggests that the integrated effect on the cardiovascular system of NMDA receptor-mediated neurotransmission is similar in both AP and mNTS, or that NMDA effectively reaches mNTS sites when delivered via the AP, or that NMDA treatment of AP neurons affects the activity of mNTS circuitry similar to direct microinjection of NMDA in mNTS.

Two problems exist in testing direct microinjection of drugs at discrete brain loci: 1) diffusion of the injected substances to adjacent sites; 2) the mechanical distortion of the injected area caused by the volume of the injectate or the pressure gradient produced during the injection (22). In these experiments we minimized these factors by using a continuous nanoliter microinfusion technique, limiting the volume injected per unit time. This minimizes the magnitude of pressure gradients produced by more rapid injection of a similar volume of injectate. We estimate that 10 nl/min represents a rate that is 1–2% of AP or NTS plasma flow based on the data and calculation of these flows by others (11). The amount of MK-801 (or NMDA) microinfused into either area postrema or mNTS was so small that its circulating concentration after removal from the brain site would yield plasma levels below threshold for any cardiovascular or neuroprotective effects (21).

We found that although MK-801 treatment of AP alone produced no significant changes in MAP and HR, these infusions inhibited baroreflex bradycardia evoked by peripheral injection of phenylephrine without affecting the tachycardia evoked by peripheral injections of nitroprusside. Mechanisms regulating heart rate in rats are both sympathetic and parasympathetic. Both sympathetic and parasympathetic tone can be significantly increased or decreased. These systems are often modulated reciprocally; however, the brainstem circuitry is neuroanatomically distinct and the identity of the various neurotransmitters or neuromodulators in each synapse of the circuitry has not been determined. Therefore, it is not difficult to understand that MK-801 injection into one discrete brainstem site might differentially affect the cardiodecelerator reflex elicited by phenylephrine challenges whereas the reflex tachycardia during nitroprusside challenge is left intact. Our results are consistent with an effect of MK-801 on circuitry within the dorsal vagal complex that can withdraw cardiac sympathetic tone and augment cardiac parasympathetic tone.

When we injected MK-801 bilaterally into mNTS it caused a significant increase in MAP and HR, but unilateral mNTS treatment with MK-801 produced neither hypertension nor tachycardia. The latter observation indicates that compensatory buffering is probably exerted by the untreated contralateral mNTS and that MK-801 does not diffuse to the contralateral mNTS. The increase in both blood pressure and heart rate when the contralateral mNTS was treated with MK-801 was very rapid. These results suggest that significant tonic modulation of both blood pressure and heart-rate regulation requires NMDA receptor-mediated neurotransmission in the mNTS under these experimental conditions. Conversely, we found that direct unilateral activation of the decelerator and depressor functions of mNTS by direct NMDA microinfusion overrode baroreflex control mechanisms. Thus, the untreated contralateral mNTS cannot buffer the effect of an ipsilateral excitatory stimulation.

Infusion of MK-801 into AP, or unilaterally into mNTS in the low-dose range used for these experiments, produced neither hypertension nor tachycardia, whereas bilateral mNTS treatment with the same dose range produced both. This suggests that the primary site at which MK-801 produces these cardiovascular effects is in the mNTS. The diffusional distance produced by AP microinfusions is either limited to the area postrema, as suggested by the control dye infusions, or at least does not diffuse far enough bilaterally to duplicate direct bilateral mNTS infusions. The result could also be consistent with the interpretation that the bilateral mNTS injections effectively treated more of the mNTS than did the AP infusions. Effective total mNTS blockade may be necessary to allow the pressor and accelerator effects to occur. Others have described similar hypertensive effects when the broad spectrum competitive glutamate antagonist, kynurenic acid, was injected bilaterally into the NTS (20).

The data also showed that pretreatment of AP with MK-801 partially blocked baroreflex bradycardia evoked by IV injection of phenylephrine. This result suggested that 1) the AP has NMDA receptor-mediated functions in common with the NTS in autonomic control of cardiovascular reflex responses, or that 2) effective blocking concentrations were achieved by diffusion from AP to mNTS that could attenuate this reflex, yet not produce hypertension or tachycardia. In support of the first interpretation, the close neuroanatomical association of AP and NTS functions is well known. AP efferent modulation of NTS activity has been suggested to be a potential pathway for AP-induced modulation of cardiovascular reflexes. Electrical stimulation of AP facilitates the effects of solitary tract activation at the level of the NTS (13). AP neurons modulate NTS neuronal activity, and this modulation results in an increase in the NTS neuronal activation (13).

In summary, NMDA and MK-801 microinfusion into both AP and mNTS affect integrated control of the cardiovascular system. This reinforces the concept that NMDA receptor-mediated neurotransmission plays an important role in the regulation of arterial blood pressure and heart rate at these sites. Both AP and mNTS receive baroreceptor afferent termi-

nals and participate in an integrative mechanism modulating outflow of autonomic activity to the periphery (1). Our results demonstrate that from a functional point of view, both AP and NTS may have NMDA receptors that are involved in the regulation of the cardiovascular system. MK-801 delivery to the brain for therapeutic management of the stroke patient will produce early cardiovascular excitatory effects by interacting at these brainstem sites. Thus, the use of NMDA antagonists for neuroprotection will involve management of cardiovascular side effects.

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