Acute Hypertension after the Local Injection of Kainic Acid into the Nucleus Tractus Solitarii of Rats

WILLIAM T. TALMAN, MARK H. PERRONE, AND DONALD J. REIS

SUMMARY Kainic acid (KA), an analogue of L-glutamate, was microinjected in 0.1 μl of saline into the nucleus tractus solitarii (NTS) of adult rats. In rats anesthetized with halothane or α-chloralose, KA injected unilaterally elicited hypotension, bradycardia, and apnea. The threshold dose was 0.1–0.2 ng (10⁻¹⁵ mol). Doses >0.2 ng blocked responses to subsequent injections for at least 30 minutes. Doses of KA >15 ng reduced the reflex bradycardia elicited by raising the arterial pressure with phenylephrine and produced arterial hypertension in rats anesthetized with α-chloralose or in other rats within 15 minutes of terminating halothane anesthesia. Bilateral injection of KA in doses >15 ng completely blocked baroreflexes and resulted in a dose-dependent elevation of arterial pressure (167 ± 9.4; P < 0.001) both in α-chloralose-anesthetized rats and in awake rats after the termination of halothane anesthesia. The hypertension rapidly led to pulmonary edema and death. Procaine microinjected also elicited fulminating hypertension; vehicle did not. Doses of KA producing hypertension caused no histological or biochemical evidence of neuronal death. The cardiovascular responses to KA were restricted to sites in the intermediate one-third of NTS and could not be elicited by injection into adjacent sites in brainstem. The results indicate that, in low doses, KA injected into NTS stimulates neurons which mediate the baroreflex, whereas, in higher doses, it produces baroreflex blockade and neurogenic hypertension. The results suggest that fulminating hypertension can be produced by nondestructive perturbations of neurochemical transmission in brain. Since the cardiovascular responses of KA are similar to those produced by microinjection into NTS of the amino acid neurotransmitter glutamic acid, the study adds further support to the hypothesis that L-glutamate is the neurotransmitter released by baroreceptor afferent nerves.

ANATOMICAL and electrophysiological studies have established that the intermediate one-third of the nucleus tractus solitarii (NTS) is the principal site of termination of the primary afferent fibers of baroreceptor nerves (Crill and Reis, 1968; Miura and Reis, 1969; Seller and Illert, 1969; Jordan and Spyer, 1977). Electrical stimulation restricted to this area simulates baroreflexes by eliciting hypotension and bradycardia (Crill and Reis, 1968; DeJong et al., 1975). On the other hand, restricted electrolytic lesions abolish baroreflexes and, in unanesthetized animals, result in neurogenic hypertension. In the rat the hypertension is fulminating (Doba and Reis, 1973; DeJong and Palkovits, 1976) whereas in the cat (Nathan and Reis, 1977) or dog (Carey et al., 1979; Schmitt and Laubie, 1979) it is chronic and labile. It is uncertain whether the hypertension produced by electrolytic lesions of NTS is a consequence of destruction of intrinsic neurons of the NTS or of interruption of the primary afferent baroreceptor fibers terminating in or passing through the region (DeJong and Palkovits, 1976).

Selective destruction of neurons without affecting axons of passage is not possible with electrolytic lesions. In recent years kainic acid (KA), a structural analogue of the putative amino acid neurotransmitter L-glutamate, has found wide use as a neurotoxin. It has been proposed (Schwarcz and Coyle, 1977a) that KA may be a useful tool for selectively destroying nerve cells without affecting fibers when locally injected into brain areas such as the striatum. KA then could be a useful tool in determining whether hypertension will result from NTS lesions which destroy neurons but spare axons.

In the present study we sought to determine the effect on arterial pressure of the local administration of kainic acid (KA) into NTS of rat. KA is known to excite most neurons in the CNS in low doses (Shinozaki and Konishi, 1970), to produce depolarization blockade in higher doses (Shinozaki and Konishi, 1970; Biscoe et al., 1976; Polc and Haeferly, 1977), and in still higher doses to be neurotoxic. It destroys cell bodies but often preserves nerve fibers when locally injected into the striatum (Schwarcz and Coyle, 1977a), the hippocampus (Nadler et al., 1978; Nadler et al., 1980), or the cerebellum (Herndon and Coyle, 1977). We shall demonstrate that low doses of KA microinjected into the NTS of rats elicit hypotension and bradycardia and that higher doses produce...
baroreflex blockade and fulminating hypertension which is not attributable to neurotoxicity.

Methods

Studies were performed on 75 adult (300-400 g) male Sprague-Dawley rats. The rats were anesthetized with halothane (2% in 100% O₂) or, after induction with halothane, with α-chloralose (20 mg/kg, iv) and then allowed spontaneously to breathe 100% O₂ administered at 1.5 liters/min via a face mask. Plastic (Tygon) cannulas were inserted into the right jugular vein and into the ventral tail artery. The arterial cannula was connected via a Statham 23Db transducer to a polygraph for continuous recording of pulsatile and mean arterial pressures (MAP) and heart rate (HR).

The animals were placed in a stereotaxic frame with the head inclined downward by 45°. An occipital craniotomy was performed. The caudal portion of the 4th ventricle was exposed by incising the dura and arachnoid and, when necessary, by retracting the cerebellum.

A glass micropipette (tip o.d. 50-100 μm) was filled with KA (2.0, 20, 50, 100, 200, or 300 ng/μl) dissolved in 0.9% saline. The pipette was placed in a micromanipulator and lowered to the calamus scriptorius, which served as the rostral-caudal (AP) and lateral (L) zero reference point. In most studies, the pipette was positioned over the intermediate portion of NTS (AP +0.4 mm; L ±0.8 mm) and lowered 0.8 mm beneath the dorsal surface of the brainstem. KA in a volume of 0.3 μl was injected at a rate of 0.3 μl/min. At the completion of each injection the micropipette was left in the brainstem for a minimum of 1 minute. It then was removed and the MAP and HR responses were confirmed visually. If no flow was observed, the injection was not discontinued, the cardiorespiratory effects would persist and the animals would die.

After completion of the injection, halothane was stopped, the wound was closed, and the MAP and HR were recorded for 15-45 minutes.

Controls were prepared similarly. In unoperated controls, no surgery was performed after insertion of cannulas. In operated controls, 0.3 μl of ascorbic acid (30 ng in saline) or 0.3 μl procaine (2%) was injected bilaterally into the NTS. Ascorbic acid (pH 3.5) was used to control for the acidity of the KA solution.

To identify sites of injection at postmortem, methylene blue was added to the injectate. The dye did not affect the physiological response to KA at active sites in NTS.

At the completion of the experiments, the animals were killed by an intracardiac injection of pentobarbital (50-75 mg). The lungs were inspected for evidence of pulmonary edema, and the ratio of lung weight:total body weight was obtained. A ratio of >1% was considered as evidence of pulmonary edema (Borison and Kovaks, 1959). The brain was removed from those animals which had received injections containing methylene blue. A transverse section of the brainstem at the level of each injection was made and the cut surface examined under a dissecting microscope to localize the stain.

The cardiovagal component of the baroreflex was tested in animals anesthetized with α-chloralose (20 mg/kg) (Talman et al., 1980a). In brief, phenylephrine (2 μg/kg in 0.1 ml) was injected intravenously as a bolus and the MAP and HR responses were recorded. The magnitude of the reflex fall in HR coincident with the maximal rise in pressure (usually 30-40 mm Hg) was determined.

Choline acetyltransferase (CAT) activity was assayed by a modification (Luine et al., 1980) of the method of Schrier and Shuster (1967). Rats were killed by decapitation and the brainstem removed and quickly frozen on Dry Ice. A transverse slice of tissue (2 mm thick) was removed by transecting the medulla 1 mm rostral and 1 mm caudal to the obex. The NTS was removed by the method of Snyder et al. (1978) and tissues were stored at -70°C until biochemical analysis was performed.

For histological examination of the brainstem, rats were anesthetized and killed by perfusion of 10% formalin through the heart. The brainstem was removed; 50-μm frozen sections were taken through the NTS and stained with cresyl violet and Luxol fast blue.

The significance of changes in arterial pressure and heart rate resulting from microinjections into the brainstem was determined by the Mann-Whitney U test (Siegel, 1956).

Results

Cardiovascular Effects of KA in the Anesthetized Rat

KA injected into the NTS unilaterally in anesthetized rats resulted in the nearly simultaneous appearance of hypotension, bradycardia, and apnea (Fig 1A). The responses appeared with administration of as little as 0.1 ng of KA, and the maximal response was elicited by 2-4 ng. (Table 1). The infusion was stopped and MAP, HR, and respiratory rate returned to baseline values (usually within 1-2 minutes). The infusion then could be completed to a total injected volume of 0.3 μl. If the infusions were not discontinued, the cardiorespiratory effects would persist and the animals would die.

The cardiovascular responses to KA could be repeated with subsequent injections until a cumulative dose of 0.2 ng had been given. At this or a higher dose, injections of KA in the ipsilateral NTS failed to produce the cardiovascular and respiratory effects for at least 30 minutes. However, a similar sequence of cardiovascular events could be elicited immediately by injections into the contralateral NTS.

Procaine (2%) or ascorbic acid (30 ng) injected
TABLE 1  
Acute Cardiovascular Effects of Kainic Acid Injected Unilaterally into the NTS of Anesthetized Rat

<table>
<thead>
<tr>
<th></th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before injection</td>
<td>After injection</td>
</tr>
<tr>
<td>Ascorbic acid (30 ng)</td>
<td>85 ± 1.6</td>
<td>85 ± 1.7</td>
</tr>
<tr>
<td>Kainic acid (4 ng)</td>
<td>88 ± 2.8</td>
<td>32 ± 4.5*</td>
</tr>
<tr>
<td>Procaine (2 × 10^3 ng)</td>
<td>96 ± 3.3</td>
<td>96 ± 3.5</td>
</tr>
</tbody>
</table>

Values expressed as the mean ± SEM were determined before and after the injection of 0.1 µl of each solution into the NTS of rats anesthetized with halothane.

* Significantly different from ascorbic acid control; P < 0.001.

unilaterally into NTS in an identical volume (0.3 µl) failed to produce any changes in MAP or HR in the anesthetized rat (Table 1).

KA injected into NTS decreased the cardiovascularg baroreflex. After a unilateral dose of 30 ng, the reflex bradycardia induced by phenylephrine was reduced to 37% of that obtained in the same rats before KA had been administered. The bilateral injection of 30 ng virtually abolished the reflex (Table 2).

Cardiovascular Effects of KA after Termination of Anesthesia

KA, when injected bilaterally into the NTS in doses of 15 ng or more, caused fulminant arterial hypertension, which developed within 15 minutes of the termination of halothane anesthesia (Fig 1B). Death occurred within a half hour. The maximum blood pressure after KA was dose-related (Fig. 2) and was significantly greater than that which occurred on termination of anesthesia in rats injected with ascorbic acid or in unoperated controls (Table 3). The threshold dose for hypertension was 6 ng and the dose which produced a maximum rise of MAP was 30 ng. In general, the HR was not significantly changed at the time when blood pressure had reached its maximum (15 min). However, later, as pulmonary edema developed, it rose dramatically (Fig. 1B; Table 3).

Hemorrhagic pulmonary edema and a lung:total body weight ratio of >1% accompanied the fulminant hypertension in all rats that had received 30 ng or more of KA into each NTS. After 15 ng of KA, only 60% of the rats developed pulmonary edema.

Hypertension (MAP = 158 ± 7.1; n = 4) also developed after termination of halothane anesthesia when KA (30 ng) had been injected into NTS unilaterally. However, blood pressure returned to normal within 2 hours, and the animals survived even after doses as high as 60 ng.

Halothane anesthesia blocked the hypertension induced by KA; however, rats anesthetized with α-chloralose (20 mg/kg) developed hypertension within 2 minutes of the completion of bilateral injections of KA (30 ng). Like KA, bilateral injection of 0.1 µl of procaine (2%) into the NTS caused hypertension in rats anesthetized with α-chloralose (Table 3).

Histological and Neurochemical Effects

We investigated whether a dose of KA producing fulminant hypertension was neurotoxic. Therefore, 60 ng of KA was injected unilaterally into NTS. Although this dose produces fulminant hypertension when injected bilaterally, it elicits only a transient hypertension when administered unilaterally. Two weeks after the injection, the rats were killed. In two animals, the brains were sectioned to examine the brainstem microscopically. In the re-

TABLE 2  Effects on Cardiovagal Baroreflex Activity of 30 ng Kainic Acid (KA) Injected in the NTS of Four Rats Anesthetized with Chloralose

<table>
<thead>
<tr>
<th></th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before PE</td>
<td>After PE</td>
</tr>
<tr>
<td>Before KA</td>
<td>95 ± 1.9</td>
<td>136 ± 2.6</td>
</tr>
<tr>
<td>After KA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>93 ± 5.3</td>
<td>128 ± 6.8</td>
</tr>
<tr>
<td>Bilateral</td>
<td>110 ± 4.3</td>
<td>140 ± 2.4</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SEM.

† Significantly different from the intra-animal control before KA (P < 0.05).
KAINIC ACID AND NEUROGENIC HYPERTENSION/Talman et al.

FIGURE 1 A: Acute hypotension and bradycardia after injection of 0.1 μl of KA into the NTS of a rat anesthetized with halothane. A control injection of 0.1 μl of ascorbic acid had no effect. B: Hypertension after injection of KA bilaterally into the NTS of a rat. Under halothane anesthesia, KA (60 ng in 0.3 μl) was injected bilaterally into the NTS and anesthesia was stopped (arrow). Note the rapid develop of hypertension and delayed tachycardia.

maintaining six animals, the area of NTS was removed and assayed for CAT, a reduction of which would be biochemical evidence of loss of cholinergic neurons (Schwarcz and Coyle, 1977a).

After the injection of 60 ng of KA there was no histological evidence of gliosis, infiltration of macrophages, cavitation, or pathological neuronal changes. The activity of CAT in the injected NTS of six rats was not significantly different from that in the homolateral NTS of six uninjected control rats (CAT activity 159 ± 23.6 nmol ACh/mg per hr in NTS of treated rats vs. 158 ± 24.0 nmol ACh/mg per hr in controls). Thus, a dose of KA sufficient to produce fulminant hypertension was not neurotoxic.

Localization of Effective Sites

By means of methylene blue staining, we located the sites of injection in 25 rats and identified the points where KA produced cardiovascular effects. For these experiments, methylene blue was added to a 300 ng/μl KA solution and 0.1 μl was injected at various locations in the brainstem. At the level of the calamus scriptorius, they were made (a) into and (b) immediately lateral to the NTS, (c) into the nucleus commissuralis, and (d) into the medial longitudinal fasciculus. At a level 0.4 mm rostral to the calamus scriptorius, they were made into (e) the medial and (f) lateral NTS, (g) the area 0.1–0.3 mm lateral to the NTS, (h) the area postrema, and (i) the external cuneate nucleus. In several additional animals, 300 ng of KA in a 1.0 μl volume were injected over 1 minute into the cisterna magna (j). Each site (a–j) was labeled five times in separate rats. No more than four injections were made in a single rat.

The location of sites from which hypotension, bradycardia, and apnea were elicited are indicated in open circles in Fig 3. Hypotension during anesthesia and hypertension after anesthesia was terminated occurred only with injections into the NTS. Injections into the area postrema, the area lateral to the NTS, the medial longitudinal fasciculus, and the external cuneate nucleus (Fig. 3)
TABLE 3  Effects on MAP and HR in the Unanesthetized Rat of the Microinjection of KA or Procaine into the NTS

<table>
<thead>
<tr>
<th></th>
<th>Unoperated control</th>
<th>Ascorbic acid (30 ng)</th>
<th>Kainic acid (30 ng)</th>
<th>Procaine (2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 9 )</td>
<td>( n = 5 )</td>
<td>( n = 5 )</td>
<td>( n = 5 )</td>
</tr>
<tr>
<td>Maximal MAP* (mm Hg)</td>
<td>118 ± 3.86</td>
<td>115 ± 2.6</td>
<td>167 ± 9.41</td>
<td>157 ± 7.2†</td>
</tr>
<tr>
<td>HR* (beats/min)</td>
<td>362 ± 8.9</td>
<td>397 ± 37.3</td>
<td>441 ± 13.1‡</td>
<td>364 ± 19.5</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SEM.
* Data represent maximal MAP and the concurrent HR within 15 minutes of termination of anesthesia.
† Significantly different from ascorbic acid and unoperated control \( (P < 0.001) \).
‡ Significantly different from unoperated control \( (P < 0.001) \) but not from ascorbic acid control.

Discussion

The present study demonstrates that KA when microinjected into the NTS of rat causes marked changes in arterial pressure, heart rate, and respiration. In the anesthetized rat the autonomic effects—hypotension, bradycardia, and apnea—resemble those elicited by natural stimulation of baroreceptors (Heymans and Neil, 1958), electrical stimulation of baroreceptor afferents (Douglas and Ritchie, 1956a, 1956b) or electrical stimulation of the NTS (Crill and Reis, 1968). The effects occur together, and cannot be attributed to mechanical distortion of NTS or to the pH of the solution, since injections of the same volume of ascorbic acid with a pH similar to that of KA did not cause changes in MAP or HR.

The hypotensive response to KA is anatomically selective and can be elicited only by injection into the intermediate one-third of the NTS. Injection intracisternally or into other regions of the medulla fails to elicit a response. Moreover, the absence of any response to injections 0.1–0.3 mm lateral to NTS, in a region traversed by some penetrating baroreceptor afferents (DeJong and Palkovits, 1976; Palkovits and Zaborszky, 1978), indicates that the response is most probably not due to diffusion of KA from NTS or to an action on axons of baroreceptor afferents which traverse the region (DeJong and Palkovits, 1976).

The hypotensive response to KA appears with as little as 0.1 ng \( (10^{-12} \text{ mol}) \) of the drug. The effect is dose dependent with the maximum response being reached with 2–4 ng. Interestingly, the response can be repeated with subsequent injections only after the lowest doses; with slightly higher doses it cannot be repeated for many minutes. This blockade of the response occurring with doses over 0.2 ng probably reflects KA's slow removal from the postsynaptic membrane (Nadler et al., 1980).

After bilateral injection of KA into NTS and cessation of anesthesia, rats develop a rapid elevation of arterial pressure to hypertensive levels. The threshold for this response (6 ng) is greater than...
that required for the hypotensive action of KA, and is well within the dose range in which repetitive stimulation of NTS by KA fails to evoke a response. The hypertensive response to KA also is dose dependent and in higher doses usually results in pulmonary edema and death.

Fulminating hypertension elicited by the local administration of KA would appear to result from pharmacologically impaired transmission in NTS for the following reasons. First, the effect is identical to that produced by destruction of NTS by electrolytic lesions (Doba and Reis, 1973). Second, KA, in doses producing hypertension, blocks the vagally mediated reflex bradycardia in response to elevation of arterial pressure by phenylephrine. Third, in rabbit, KA microinjected into NTS blocks the postsynaptic response evoked in NTS by electrical stimulation of the baroreflex on sympathetic renal nerve activity evoked by electrical stimulation of the medullary pressor area (Reis et al., in press). Fourth, the injection of procaine, a local anesthetic, into NTS elicits fulminating hypertension which is, of note, not preceded by hypotension.

KA, when injected into other brain areas, destroys neurons and even fibers (Schwarcz and Coyle, 1977a; Zaczek et al., 1978), but its effects on blood pressure in rat in this study cannot be attributed to neurotoxicity. The dose of KA required to elicit hypertension was orders of magnitude less than that needed to produce cellular destruction in such vulnerable areas as retina (Schwarcz and Coyle, 1977b), hypothalamus (Olney et al., 1975), striatum (Schwarcz and Coyle, 1977a), hippocampus (Nadler et al., 1978), and cerebellum (Herndon and Coyle, 1977). For example, the minimum neurotoxic dose in the striatum is approximately 10^-8 mol. In contrast, the minimal effective dose for producing hypertension in this study was 15 ng (10^-10 mol), and doses of 60 ng injected into the NTS unilaterally did not produce any histological or biochemical evidence of cellular damage.

The most reasonable interpretation of our findings can be related to the pharmacology of KA. Others have established by electrophysiological and iontophoretic techniques that in the lowest doses KA, like its analogue L-glutamate, excites neurons (Shimozaki and Konishi, 1970), but in higher doses it produces a depolarization blockade (Biscoe et al., 1976; Polc and Haefely, 1977). Only in the highest doses is it neurotoxic (Schwarcz and Coyle, 1977a). Thus in this study the baroreflex-like response evoked by injecting KA into NTS can be interpreted as resulting from excitation of intrinsic NTS neurons which mediate baroreflexes. At the higher doses KA produced functional blockade of baroreflexes attributable to a depolarization blockade of the same NTS neurons.

There are several implications of this study. First, it demonstrates that KA injected bilaterally into NTS results in fulminating hypertension which is identical to that produced in rats by bilateral electrolytic lesions of NTS. This finding indicates that the hypertension produced by the electrolytic lesions does not rely on destruction of baroreceptor afferents projecting into the NTS as proposed (DeJong and Palkovits, 1976). Rather, it can be entirely a consequence of impaired function of intrinsic neurons in NTS.

Second, the findings raise interesting questions with respect to the identity of the neurotransmitters of baroreceptor nerves. KA is generally believed to mimic the effects of L-glutamate, although it is uncertain whether the effects are mediated via receptors for L-glutamate or another class of receptors sensitive to KA (London and Coyle, 1979a, 1979b). Since KA is pharmacologically like L-glutamate, a naturally occurring neurotransmitter, it suggests that L-glutamate may be the neurotransmitter released within NTS from baroreceptor afferent fibers. Consistent with the hypothesis are our recent observations that: (1) the microinjection of L-glutamate into NTS, like KA, elicits a dose dependent baroreceptor-like response in anesthetized rats (Talman et al., 1980b); (2) high doses of L-glutamate block baroreflexes and in unanesthetized rats, produce hypertension (Reis et al., in press); and (3) unilateral removal of the nodose ganglion, the site of the cell bodies of origin of many baroreceptor afferents, results in a biochemical and anatomically selective reduction (by over 60%) of the high affinity uptake of L-glutamate into synaptosomal preparations of NTS (Talman et al., 1980b). Thus KA may produce its action on blood pressure by mimicking the action of the natural transmitter L-glutamate.

Finally, the study indicates that arterial hypertension can be produced by interference with chemical neurotransmission through a selective brain area. It thus establishes the possibility that a local chemical imbalance in brain can result in hypertension.

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