Ganglionic Blockade With Tetraethylammonium in Conscious Rats

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The sympathetic ganglionic blocking agent tetraethylammonium has been used as a clearance marker for the measurement of renal plasma flow, but the sympathetic ganglionic blocking dose in rats is unknown. In light of differential reflex activation of sympathetic nerve activity to heart and kidney, we compared the effect of tetraethylammonium on renal nerve activity, mean arterial pressure, and heart rate. Conscious rats were infused with either vehicle (isotonic saline) or tetraethylammonium \((n=7\) in both groups). Tetraethylammonium was infused cumulatively (35 minutes per dose) in the following doses: \((10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, \text{ and } 10^{-1})\) g/kg body wt per hour. Doses for 15% reduction were \((10^{-1})\) for mean arterial pressure, \((0.55 \times 10^{-1})\) for heart rate, and \((0.055 \times 10^{-1})\) g/kg body wt per hour for renal nerve activity. Renal nerve activity was abolished at \((10^{-1})\) g/kg body wt per hour; mean arterial pressure and heart rate were unchanged at doses lower than \((10^{-1})\) g/kg body wt per hour. The lethal dose was \(1\) g/kg body wt per hour. No changes were observed in vehicle-treated animals. Tetraethylammonium at \((10^{-1})\) g/kg body wt per hour resulted in an attenuated increase in renal nerve activity during acetylcholine-induced reduction in mean arterial pressure, reflecting arterial baroreceptor inhibition. We conclude that renal nerve activity is 10- and 18-fold more sensitive to sympathetic ganglionic blockade than heart rate and mean arterial pressure, respectively. When tetraethylammonium is used as a clearance marker for measurement of renal plasma flow in rats, it should be administered in doses less than \((10^{-2})\) g/kg body wt per hour. (Hypertension 1992;19:814-817)

KEY WORDS • tetraethylammonium compounds • blood pressure • renal nerves • heart rate • ganglionic blockers

The sympathetic ganglionic blocking agent tetraethylammonium (TEA) has been proposed as a clearance marker for measurement of renal plasma flow. In contrast to the most commonly used marker of renal plasma flow, \(p\)-aminohippurate (PAH), the renal extraction of TEA is unaltered during conditions of azotemia or administration of anionic drugs. This has been ascribed to the fact that PAH is secreted by the organic anion transporter in the proximal tubules. Therefore, because of competitive inhibition in the organic anion pathway, tubular secretion of PAH is decreased during conditions of accumulation of exogenous or endogenous anions. Furthermore, during intravenous infusion of dextrose in rats, the renal extraction of PAH is decreased, whereas dextrose infusion does not affect the renal extraction of TEA. However, because of its sympathetic ganglionic blocking effect, TEA is not an ideal marker for renal plasma flow, and therefore, during conditions with increased renal nerve activity (RNA), e.g., hypertension, the renal effects of increased renal sympathetic outflow could be masked by the ganglionic blocking effect of TEA. This problem has been approached by administration of radioactively labeled TEA, which allows accurate determination of TEA clearance at low plasma TEA concentrations. In all previous studies in which tracer amounts of TEA have been administered, the sympathetic ganglionic blocking effect has been assumed to be negligible; however, the sympathetic ganglionic blocking dose of TEA in rats is unknown. Therefore, the aim of this study was to examine the dose–response relation of TEA for heart rate (HR), mean arterial blood pressure (MAP), and RNA.

Methods

Animals
Experiments were performed in 14 male Sprague-Dawley rats (318-358 g; Harlan Sprague-Dawley, Inc., Indianapolis, Ind.). All animal procedures were in accord with guidelines for animal use at the University of Iowa.

Anesthesia
Rats were anesthetized with methohexital (Brevital, 20 mg/kg i.p. followed by 10 mg/hr i.v. after implantation of the venous catheter; Eli Lilly, Indianapolis, Ind.).
Surgical Procedures

Polyethylene catheters (PE-50, Clay Adams, Parsippany, N.J.) were implanted in the right jugular vein and carotid artery. The left kidney was exposed through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope (×25), a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a bipolar platinum wire electrode (Cooner Wire Co., Chatworth, Calif.). RNA was amplified (×10,000-50,000) and filtered (low, 30 Hz; high, 3,000 Hz) with a P511 bandpass amplifier (Grass Instrument Co., Quincy, Mass.). The amplified and filtered signal was channeled to a 5113 oscilloscope (Tektronix Inc., Beaverton, Ore.) and Model R611 polygraph (Beckman Instruments, Schiller Park, Ill.) for visual evaluation, to an audio amplifier-loudspeaker (model AM6 Audio Monitor, Grass) for auditory evaluation, and to a rectifying voltage integrator (model 9873B, Beckman). The integrated voltage and renal neurogram signals were displayed on the Beckman polygraph. We assessed the quality of the RNA by its pulse-synchronous rhythmicity and by examining the magnitude of decrease in recorded RNA during sinoaortic baroreceptor loading with an intravenous injection of 2 μg norepinephrine. The RNA remaining after maximum inhibition after norepinephrine administration was similar to the background noise observed approximately 30 minutes postmortem; this value was subtracted from all experimental values of RNA. When an optimal RNA was observed, the recording electrode was fixed to the renal nerve branch with a silicone cement (Wacker Sil-Gel 604, Wacker-Chemie, Munich, FRG). Then the electrode cable was secured to the abdominal trunk muscles. Finally, the electrode cable was exteriorized, and the flank incision was closed in layers.

Experimental Protocol

After surgery, intravenous infusion with isotonic saline (20 μl/min) was started, and the rats were allowed to recover from anesthesia and surgery. Blood pressure and HR were measured continuously by Statham P23Db pressure transducers coupled to the Beckman polygraph. HR was recorded with a linear cardioactometer (Beckman 9857B) triggered by the arterial pressure waveform. To avoid noise in the renal nerve recording signal due to motion during acetylcholine administration, the rats were given an infusion of a sedative dose of diazepam (20 μg/kg body wt per minute; Diazepam, Elkins-Sinn Inc., Cherry Hill, N.J.). After 3 hours, the intravenous injection of 2 μg norepinephrine was repeated. Experiments were included if the RNA response to norepinephrine was similar to that previously observed at the time of implantation of the electrode. To assess the RNA response to arterial baroreceptor unloading before TEA treatment, we reduced the arterial pressure to 50-60 mm Hg by infusion of acetylcholine (0.5-3.0 mg/min; acetylcholine chloride, Sigma Chemical Co., St. Louis, Mo.) for 30-60 seconds. Rats were randomized to treatment with TEA (tetraethylammonium bromide, Sigma) or vehicle (isotonic saline). TEA was administered cumulatively as a priming dose (one fifth of hourly dose) followed by continuous intravenous infusion in the following doses: 0 (control), 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ g/kg body wt per hour. In pilot studies, the lethal dose was found to be 1 g/kg body wt per hour. After administration of the priming dose, MAP, HR, and RNA were allowed to stabilize during a 20-minute equilibration period, after which RNA was recorded for 15 minutes. After the last treatment period, the RNA response to arterial baroreceptor unloading was assessed as before TEA treatment by acetylcholine administration. At the end of the experiment, the quality of the renal nerve signal was again evaluated with an intravenous injection of norepinephrine (2 μg). The rat was then killed by an intravenous overdose of pentobarbital (25 mg; Nembutal, Abbott Laboratories, Chicago), and RNA was continuously recorded for a further 30 minutes as a measure of background noise.

Statistical Analysis

Statistical analyses were conducted with repeated-measures analysis of variance followed by Student's t test with Bonferroni's correction for multiple comparisons. Differences were considered statistically significant at a value of p<0.05. All values presented are mean±SEM.

Results

Figure 1 shows the dose-dependent effects of TEA on MAP, HR, and RNA. There were no differences in MAP between groups, but MAP was significantly reduced by 10⁻¹ g TEA/kg body wt per hour, and it was unchanged in vehicle-treated animals. The effect of TEA on HR showed the same dose dependency as MAP since HR was only reduced at 10⁻¹ g/kg body wt per hour. In contrast to MAP and HR, RNA was significantly reduced at 10⁻² g/kg body wt per hour, but it remained constant in vehicle-infused animals throughout the experiment. The average RNA corrected for the background signal was 39±3 integrated RNA units per minute during the control period, and it was not significantly different between the two groups. Figure 2 shows plots of the dose-dependent relative changes in MAP, HR, and RNA during TEA administration. The doses for 15% reduction were 10⁻¹ for MAP, 0.55±10⁻¹ for HR, and 0.055±10⁻¹ g/kg body wt per hour for RNA. Thus, RNA was sensitive to sympathetic ganglionic blockade with TEA at 10⁻¹ and 18-fold lower doses than HR and MAP, respectively. Figure 3 shows the responses of RNA to acetylcholine administration before and after TEA treatment. The acetylcholine-induced increase in RNA was the same in both groups before treatment. Although the RNA response remained unchanged in vehicle-treated animals, it was only 60±16% of control in rats infused with 10⁻¹ g TEA/kg body wt per hour. However, acetylcholine still increased RNA in rats infused with 10⁻¹ g TEA/kg body wt per hour, suggesting that the sympathetic ganglionic blockade was not complete. During acetylcholine administration, MAP was reduced to the same level before and after TEA treatment in both groups (Figure 4).

Discussion

This study shows that TEA blocks sympathetic ganglionic neurotransmission effectively in rats at 10⁻¹ g/kg.
body wt per hour. However, at $10^{-2}$ g/kg body wt per hour, a modest reduction in RNA was observed, suggesting that when TEA is used as a clearance marker for the measurement of renal plasma flow in rats, it should be administered in doses less than $10^{-2}$ g/kg body wt per hour. In comparison, the sympathetic ganglionic blocking dose is $10^{-2}$ g/kg body wt in cats and dogs. In studies in which TEA has been given as a constant infusion with carbon-14-labeled TEA, the infusion dose was approximately $0.5 \cdot 10^{-3}$ g/kg body wt per hour. Therefore, when tracer doses of carbon-14-labeled TEA are used in rats, TEA does not affect cardiovascular or renal function by blockade of ganglionic neurotransmission.

**FIGURE 2.** Line plot shows dose-dependent relative changes in mean arterial pressure (MAP), heart rate (HR), and renal nerve activity (RNA) during infusion with tetraethylammonium (TEA). C, control; b.w.h, body weight per hour.

**FIGURE 3.** Bar graph shows effects of acetylcholine (Ach)-induced reduction of mean arterial pressure on renal nerve activity. Response was evaluated both before and after treatment with vehicle or tetraethylammonium (TEA) ($10^{-1}$ g/kg body wt per hour). C, control. *p<0.05; ns, nonsignificant difference.

**FIGURE 4.** Bar graph shows effects of short-lasting acetylcholine (Ach) infusion (0.5–3.0 mg/min for 30–60 seconds) on mean arterial pressure. Acetylcholine was given both before and after treatment with vehicle or tetraethylammonium (TEA) ($10^{-2}$ g/kg body wt per hour). C, control. *p<0.05; ns, nonsignificant difference.
During reflex activation of efferent sympathetic nerve activity, the peripheral responses are differentiated because of central modification by reflex interactions, depending on the nature of reflex activation. The higher sensitivity to sympathetic ganglionic blockade of RNA compared with HR is in agreement with the view that the kidney, in contrast to the heart, lacks parasym pathetic innervation. Thus, multifiber RNA is a better estimate of peripheral sympathetic nerve activity than HR. In accord with the cohesive integration of the multiple mechanisms involved in control of arterial pressure, MAP was the least sensitive parameter to sympathetic ganglionic blockade.

In conclusion, effective sympathetic ganglionic blockade in conscious rats is achieved by administration of $10^{-1}$ g TEA/kg body wt per hour. However, in doses less than $10^{-2}$ g/kg body wt per hour, TEA exerts no effects on MAP, HR, or RNA. The higher sensitivity to sympathetic ganglionic blockade of RNA compared with HR and MAP is in accordance with the predominance of noradrenergic sympathetic fibers in the renal nerves, whereas other mechanisms modify the effect of sympathetic ganglionic blockade on HR and MAP.

References
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