Pharmacologic Actions of Tetrodotoxin
Studied by Direct Perfusion of the Sinus Node

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ABSTRACT

The effects of tetrodotoxin on sinus node activity were studied by direct perfusion through its nutrient artery. Tetrodotoxin suppresses action potentials by blocking sodium entry into the cell. In the open-chest anesthetized dog, direct perfusion of the sinus node artery with tetrodotoxin, 1.0 µg/ml, caused immediate sinus slowing lasting 12 to 60 minutes. This slowing was not affected by intranodal atropinization. Tetrodotoxin also produced complete blockade of sinus node response to vagal stimulation. Blockade of sinus node responses to stellate stimulation was less profound. Tetrodotoxin had no effect on chronotropic responses to intranodal acetylcholine and norepinephrine. These findings suggest that blockade of transmembrane sodium transport (or the rapid entry channel) has a negative chronotropic effect which is not cholinergic and during which response to neurotransmitter substances is normal. Concomitant with this effect there is blockade of neurotransmitter release (or synthesis) in the sinus node.

ADDITIONAL KEY WORDS
chronotropic effect of tetrodotoxin
sodium channel cholinergic mechanisms of sinus node
adrenergic mechanisms of sinus node
anesthetized dog

Tetrodotoxin has become a useful experimental tool because it blocks action potentials in certain excitable cells through a selective effect on early transient (sodium) conductance (1-4). It does not block action potentials in membranes maintaining excitability in sodium-free media (barnacle muscle [5] and guinea pig tenia coli [6, 7]), nor does it block generator potentials (Pacinian corpuscles and crayfish stretch receptors [8]).

Several observations of cardiac effects of tetrodotoxin administered systemically have been published (9-15). However, the profound extracardiac effects of this neurotoxin obscure its direct cardiac effects. The development of an experimental preparation permitting direct perfusion of the canine sinus node through its own artery (16) permits a study of the direct effects of tetrodotoxin on the sinus node in situ and of its normally preserved innervation. Because nodal perfusion was direct, sufficiently small quantities of test substances could be used so that systemic effects on recirculation were negligible.
Methods

Nineteen dogs were anesthetized with pentobarbital sodium, 30 mg/kg iv, and the trachea was intubated for mechanical ventilation with room air. With the chest opened in the midline and the heart suspended in the pericardium, the right coronary artery was dissected free and ligated at the site of origin of the sinus node branch. After its ligation, the right coronary artery was opened distal to the tie and a small polyethylene cannula was inserted into the sinus node branch and ligated in place. Collateral circulation to the canine sinus node is so extensive (17) that ligation of its primary arterial supply has no significant effect on sinus rhythm. Details of the method have been previously reported (16, 18). A three-way stopcock attached to the proximal end of the cannula in the sinus node artery permitted injections into the artery. Cannulas were also placed in the right atrium via the jugular vein and in the central aorta via a femoral artery. Transducer measurements of pressures in the right atrium, sinus node artery (retrograde), and central aorta, along with an instantaneous tachogram and the ECG, were recorded on a direct-writing polygraph at 0.25 mm/sec. The pulses and the ECG (AVR) were monitored on a multitrace oscilloscope at a sweep speed of 50 mm/sec. A separate one-channel ECG (either AVR or a wick electrogram from the sinus node) was recorded simultaneously at 25 mm/sec and collated with the polygraph. The right vagus nerve was isolated in the neck, and the right stellate ganglion was isolated within the thorax for stimulation at supramaximal voltage, 32 cps, with 1-msec rectangular impulses in 6-second trains.

Crystalline tetrodotoxin (Sankyo Co., Tokyo, Japan) was dissolved in glass-distilled water and stored at 4°C. Test materials were prepared fresh in Ringer's solution and were always compared with control injections of Ringer's solution alone. All injections were from a hand syringe and usually of 2-ml volume delivered in 5 to 10 seconds. Solutions for injection were kept in a constant-temperature water bath at 38 to 39°C. Any injection into the sinus node artery produces a transient bradycardia during the injection followed by a brief acceleration after injection. The bradycardia is not due to abnormal temperature, pH, or ion or oxygen content of the injectate since it occurs with fresh warm autogenous arterial blood. It is probably a physical response to distention of the sinus node artery (18). The acceleration after injection is due to a transient release of local nodal norepinephrine (19).

Results

Concentrations of tetrodotoxin from $10^{-8}$ to $10^{-4} \mu g/ml$ had no direct chronotropic effect and did not affect chronotropic responses to stellate or vagal stimulations. At 1.0 $\mu g/ml$ there was immediate sinus slowing in all the dogs, $26 \pm 10$ beats/min (mean $\pm 1$ sd) below control heart rate. Following the immediate onset of bradycardia, there was a further decline in heart rate. Minimum heart rate was obtained 2 to 6 minutes after injection, and then the rate gradually returned to control levels (Fig. 1). Duration of this negative chronotropic effect was 12 to 60 minutes.
Figure 2

Right atrial wick electrogram recorded at 25 mm/sec. Responses in the same dog, from above down, are as follows. A control right stellate (RS) stimulation is followed by sinus acceleration to 192 beats/min. The rate increases to a maximum of 228 beats/min following intranodal norepinephrine (NE). There is complete sinus arrest during right vagus (RV) stimulation. Simultaneous RV stimulation and injection of tetrodotoxin (TTX), 1.0 μg/ml, into the sinus node artery shows rapid blockade of vagal effects on the sinus node, the P wave returning within 3 seconds of beginning the injection. Subsequently, the acceleration response to RS stimulation is also blocked, but the response to intranodal NE is not affected. A repeat RV stimulation has no effect on sinus rate but produces complete A-V block.

Tetrodotoxin, 1.0 μg/ml, produced complete blockade of the response to vagal stimulation (Fig. 2). In experiments in which the injection into the sinus node artery was begun simultaneously with vagal stimulation, the blockade was always evident within 2 to 3 seconds of beginning the injection, indicating a very rapid onset of action. Vagal responses gradually returned to control level within 25 to 60 minutes. Blockade of the response to stellate stimulation after injection of tetrodotoxin, 1.0 μg/ml, into the sinus node artery was less profound (Figs. 2 and 3) than vagal blockade. Two of 10 dogs had complete blockade of stellate acceleration of the sinus node, and two others showed no
Tetrodotoxin, 1.0 µg/ml, is injected into the sinus node artery following control responses to right stellate (RS) stimulation and intranodal norepinephrine (NE). The stellate response is diminished but not completely blocked while the response to intranodal norepinephrine is unaffected. Stellate responsiveness gradually returns; in this dog there was recovery to control stellate response in 55 minutes. The asterisks indicate periods of transient A-V nodal rhythm apparent in the right atrial (RA) pressure trace when the stellate effect on the A-V node permits it to assume pacemaking from the sinus node. Artifact is seen in the ECG-derived tachogram.

**FIGURE 3**

Evidence of blockade of stellate response. Average peak acceleration with stellate stimulation following tetrodotoxin, 1.0 µg/ml, was 47.8% of the control average. Recovery to control accelerative responses (in the eight dogs with partial or complete blockade) took 12 to 55 minutes. Although it produced adrenergic and cholinergic neural blockade, injection of tetrodotoxin, 1.0 µg/ml, into the sinus node artery had no effect on responses by the sinus node to direct perfusion with either acetylcholine, 0.1 to 1.0 µg/ml, or norepinephrine, 0.1 µg/ml (Fig. 3).

**Discussion**

Low concentrations of tetrodotoxin (10⁻⁸ to 10⁻⁷ g/ml) block action potentials in squid giant axon (2), eel electroplaques (21), frog skeletal muscle (1), and many other excitable cellular membranes. The effective concentration of tetrodotoxin in our experiments was 1 x 10⁻⁶ g/ml (1.0 µg/ml). Two factors can account for this difference: (1) Our test solution is injected over a period of only 5 to 10 seconds; (2) The heart is relatively resistant to the effects of tetrodotoxin (22). Sano et al. (14) found that sinus node action potentials were preserved in tetrodotoxin concentrations of 10⁻⁶ or 10⁻⁵ g/ml.

Slowing of heart rate with systemic tetrodotoxin has been reported in the cat and dog (13) and in the rabbit, guinea pig, pig, rat, and mouse (15). In other reports (11) no cardiac slowing was noted, perhaps because of the effect on the sinus node of catecholamines released secondary to the profound hypotension of systemic intoxication. The mechanism of this sinus slowing is uncertain, since tetrodotoxin has little effect on...
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the form of cardiac action potentials (22) except for reduction of the rate of rise of the action potential (frog ventricle) (9). The failure of nodal atropinization to affect the slowing response to tetrodotoxin in our experiments suggests that it is not a cholinergic mechanism.

Although decreased resting adrenergic influence may partially explain bradycardia from tetrodotoxin, the lesser effect on stellate stimulus response and the normally preserved response to directly administered noradrenaline (and presumably to circulating catecholamines) seem more compatible with a direct negative chronotropic effect by tetrodotoxin on pacemaking cells. This would support the postulate of Trautwein and Kassem (23) that one of the important mechanisms underlying the spontaneous heart beat is a high resting sodium conductance. In their experiments, pacemaker activity in the heart was enhanced by conditions which increased sodium conductance; we have found that pacemaker activity is depressed during the period of decreased sodium conductance produced by tetrodotoxin.

Failure of tetrodotoxin to affect the chronotropic responses to intranodal acetylcholine or norepinephrine is consistent with observations reported by Kao and Fuhrman (11), who found in cat experiments that tetrodotoxin, 1.4 to 3 μg/kg iv, did not prevent cardiac arrest with acetylcholine, and the pressor effects of epinephrine and norepinephrine were preserved. More recently Toda (24) has reported that decreased external sodium concentration augmented the response of isolated sinus node of rabbits to stimulation of their attached sympathetic nerves. However, this phenomenon depended on the stimulus rate; it was distinct at 5/sec and much less apparent at 1 or 20/sec, and its significance relative to our own findings is presently uncertain.

Postganglionic adrenergic nerves have been reported to respond more slowly and to a lesser degree than preganglionic fibers (11). In our experiments cholinergic neural blockade by tetrodotoxin was always complete, while adrenergic neural blockade was often partial. Cholinergic ganglion cells (and their associated preganglionic endings) are abundant near the margins of the sinus node (25), placing the preganglionic cholinergic fibers within the zone perfused in our experiments. Whether a preganglionic effect by tetrodotoxin in our experiments was an important factor or not, there seems little doubt that neurotransmitter release (or synthesis) at postganglionic terminals in the sinus node was impaired during the sinus bradycardia.

References

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