Mechanism of Cardiovascular Action of Tetrodotoxin in the Cat

BLOCK OF CONDUCTION IN PERIPHERAL SYMPATHETIC FIBERS

By Maurice B. Feinstein, Ph.D., and Maxine Paimre, B.S.

ABSTRACT

Tetrodotoxin given to cats in doses of 1 to 10 μg/kg iv caused profound cardiovascular depression characterized by decreases in systolic and diastolic blood pressures, pulse pressure, heart rate, force of myocardial contraction, cardiac output and peripheral resistance. These effects were associated with marked inhibition of the responses to stimulation of nerves to the adrenal medulla and the sympathetic nerves to heart and blood vessels. Sinus bradycardia due to toxin did not occur after acute cardiac sympathetic denervation. In isolated sympathetic nerve-right atrium preparations, tetrodotoxin at 1 to 2 x 10⁻⁸ g/ml abolished the positive inotropic and chronotropic responses to nerve stimulation without effect on spontaneous rate or force, or transmitter release by tyramine. Toxin-produced vasodilation in perfused hindlimbs was associated with marked inhibition of the normal vasoconstrictor responses to sympathetic vasomotor nerve stimulation. Vasodilation was not due to a direct action on vascular smooth muscle, since it was not seen after α-receptor blockade with phenoxybenzamine, after sympathetic denervation, or after blockade of adrenergic transmitter release by guanethidine or β-TM 10 accompanied by adrenalectomy. Vasodilators such as acetylcholine, histamine and isoproterenol consistently produced additional vasodilation under these conditions. The hypotensive effect of the toxin was also observed in spinal cats. It is concluded that the potent hypotensive action of tetrodotoxin, in the cat, is primarily due to blockade of conduction in peripheral sympathetic nerves and nerve fibers innervating the adrenal medulla.

ADDITIONAL KEY WORDS

myocardial contraction  vasomotor center  cardiac output

spinal cat

Tetrodotoxin is the purified active principle responsible for tetrodon poisoning, also known as puffer-fish or fugu poisoning. It has become a very important tool in the study of excitation phenomena in cells because of its potent and highly selective inhibition of the initial changes in membrane permeability responsible for the spike potential in nerve (1). The toxin's poisonous properties in man and other animals stem from its depressant actions on the cardiovascular and respiratory systems, thus leading to severe hypotension and respiratory paralysis. The mechanism of the hypotensive action of tetrodotoxin remains a subject of controversy, although reports on its circulatory effects have appeared since 1890. The hypotension has been ascribed variously to depression of central vasomotor areas (2-5), a histamine-like vasodilator action (4), a direct vasodilator action on vascular smooth muscle (6), ganglionic or preganglionic blockade (7), and blockade of vasomotor nerves (8). Evidence for and against brain vasomotor centers as the principal site of toxin action has recently been published (5,9).
It has been rather generally concluded that the cardiovascular effects of tetrodotoxin result from a loss of vasomotor tone without any significant direct action on the heart (1, 5). However, the evidence concerning the effects of the toxin on the mammalian heart is meager and often contradictory. Although Kao and Fuhrman (7) reported little if any alteration of the heart rate in cats, except at very high doses of about 20 μg/kg, Murtha and associates (3) observed some decrease in heart rate in dogs receiving doses of only 3 to 6 μg/kg and a substantial decrease in myocardial contractility. The method by which the latter effect was studied was not described, and little attention has been paid since then to the actions of the toxin on the mammalian heart. Tsukada (10) found that tetrodotoxin produced a slight sinus bradycardia in rodents which was not prevented by vagotomy or atropine. Conduction disturbances between atrium and ventricles have also been described (2, 8, 10).

In this paper we report the effects of tetrodotoxin on the responses of the cat to stimulation of sympathetic fibers that innervate the heart, blood vessels, and adrenal medulla. We found that peripheral vasodilation and depression of myocardial function result from the potent blockade of conduction in these peripheral sympathetic nerves by tetrodotoxin, thus confirming the early conclusion of Ishihara (8), which was based solely on visual observations of blood vessels.

Methods

Cats of both sexes, ranging in weight from 1.8 to 2.5 kg, were anesthetized either by intraperitoneal injection of sodium pentobartital, 35 mg/kg, or by ether followed by intravenous injection of α-chloralose, 40 mg/kg, after which the ether was discontinued. All animals were artificially ventilated because of the toxin’s ability to produce respiratory paralysis. In five cats, no pentobarbital was given; the spinal cord was sectioned at C1 under ether anesthesia. The ether was then discontinued, and the animals were artificially ventilated at a minute volume of about 0.3 liters with a respirator (E & M Instrument Co. model V5KC) equipped with a non-rebreathing valve. The body temperature was maintained by warming the animals with lamps.

VASCULAR PRESSURES, VENTRICULAR FORCE AND CARDIAC OUTPUT

Blood pressures were recorded on a Grass model 5D polygraph from either the carotid or femoral arteries with Statham P23D pressure transducers. Pulse rates and electrocardiograms were monitored with a Grass SP4 preamplifier. Intraventricular pressures were measured by direct needle puncture of the left ventricle after sternal thoracotomy. A Walton-Brodie strain gauge arch, modified in size for the cat heart (11), was used to assess the force of ventricular contraction from a segment of the myocardium 5 to 6 mm long. The strip of ventricle between the feet of the arch was stretched by approximately 50% as recommended by Cotten and Bay (12) to minimize errors due to changes in the size of the ventricles.

Cardiac output was determined by the dye dilution method, with indocyanine green1 as the indicator. The dye was injected into the jugular vein, and arterial blood was withdrawn from the carotid artery at a constant rate by an infusion-withdrawal pump (Harvard Apparatus Co.) through a flow cell2 placed in a Beckman DU spectrophotometer. The dye concentration curves in the blood were recorded with a Gilford model 2000 recorder at 800 mmfi, with indocyanine green 1 as the indicator. The dye was injected into the jugular vein, and arterial blood was withdrawn from the carotid artery at a constant rate by an infusion-withdrawal pump (Harvard Apparatus Co.) through a flow cell2 placed in a Beckman DU spectrophotometer. The dye concentration curves in the blood were recorded with a Gilford model 2000 recorder at 800 mmfi, after which the withdrawn blood (about 10 to 15 ml) was rein fused. The response time of the recorder was 1 second for full scale (10-inch chart) deflection. Cardiac output was calculated from the curves after correction for recirculation of the dye by the method of Kinsman and associates (13). Duplicate determinations of the control cardiac outputs in each cat agreed within 10%. The control cardiac output in seven experiments was 0.346 liters/min (± 0.030 se), which agrees favorably with a mean value of 0.33 liters/min previously reported for cats (14, 15).

HINDLimb perfusion

Blood flow in the abdominal aorta, just below the inferior mesenteric artery, was diverted through a polyethylene cannula to a 5-ml reservoir heated to 37°C, and then into a Holter perfusion pump. The blood was returned by the pump at a controlled constant rate back into the aorta through a second cannula inserted just rostral to the bifurcation of the external iliac arteries. The perfusion pressure was monitored by a Statham P23A transducer through the side-arm of a T-tube, inserted into the outflow cannula from the pump. Under constant flow cond-

1Cardigreen, donated by Hynson, Westcott & Dunning, Baltimore, Md.
2Hellma Cells Inc., Jamaica, N. Y., cell No. 137 (0.3 ml).
CARDIOVASCULAR ACTIONS OF TETRODOTOXIN

The effects of tetrodotoxin on the responses to stimulation of various pre- and postganglionic autonomic nerves were investigated after surgical isolation of the respective nerve trunks and ganglia. The nerves were placed over platinum wire electrodes and kept moist and electrically insulated from the surrounding tissues by warm mineral oil. Stimulation at supramaximal voltage, 2-msec duration, and frequencies varying from 5 to 35 impulses/sec was provided by a Grass stimulator. Among the nerves stimulated and the corresponding physiologic responses elicited thereby were: (1) pre- and postganglionic fibers of the superior cervical ganglion (contraction of the nictitating membrane); (2) pre- and postganglionic fibers of the right and left stellate ganglia (increased arterial blood pressure, intraventricular pressure, heart rate, and ventricular force); (3) splanchnic nerve fibers or fibers from the celiac ganglia that innervate the adrenal medulla (increased blood pressure and heart rate); (4) pre- and postganglionic fibers of the lumbar sympathetic chain and postganglionic fibers running in the sciatic nerve trunk (increased vascular resistance in the perfused hind limbs and tail); (5) right cervical vagal trunk (decreased blood pressure and heart rate). In two experiments, a vasopressor area located in the sigmoid gyrus of the cerebral cortex was electrically stimulated after trephining the skull.

ISOLATED SYMPATHETIC NERVE-ATRIUM PREPARATIONS

Under artificial respiration, the chest was opened by splitting the sternum. The right thoracic sympathetic chain and the stellate ganglion were isolated. These were dissected out along with the entire heart and the tissues through which the postganglionic cardiac nerves course to the heart. The dissection was continued in a culture dish containing Krebs solution perfused with 95% O₂-5% CO₂ so as to remove as much extraneous tissue as possible, including the left atrium and both ventricles. The pre- and postganglionic fibers were placed over two pairs of platinum electrodes held by micromanipulators so that they could be lifted out of the bath momentarily during the period of stimulation. The right atrium was fixed in a horizontal position and its force of contraction measured with a Grass FT 03 transducer. In some experiments, electrical activity of the atria was recorded with a suction electrode (17). This method measures the demarcation or injury potential, which is a variable fraction of the actual membrane potential. The magnitude of the action potentials measured in our experiments ranged from 5 to 30 mv. The configuration of the action potential is almost exactly the same as that obtained with intracellular microelectrodes (18), and a good implantation of the suction electrode enables one to record from a contracting atrium of a cat for considerable periods without the misfortune of electrode breakage or displacement, which is so common in this tissue when using glass microelectrodes.

Crystalline tetrodotoxin was obtained from the Sankyo Co., Tokyo, Japan.

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The composition of the solution (in mM) was:
NaCl 120, KCl 4.6, CaCl₂ 2.6, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, glucose 11.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Cardiovascular Effects of Tetrodotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>No. of experiments</td>
<td>36</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>183 ± 5</td>
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<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>132 ± 4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>94 ± 3</td>
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<tr>
<td>ECC intervals (msec)</td>
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<tr>
<td>Q-T</td>
<td>249 ± 7</td>
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<tr>
<td>Q-S</td>
<td>68 ± 2</td>
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<tr>
<td>P-R</td>
<td>72 ± 2</td>
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<tr>
<td>P-P</td>
<td>341 ± 11</td>
</tr>
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</table>

Values are mean ± SEM. 
*P > .05; for all other values P < .01. Calculations by Student's t-test.
RESULTS

EFFECTS OF TETRODOTOXIN ON BLOOD PRESSURE, HEART RATE, ECG, AND PULSE WAVES

The principal cardiovascular actions of tetrodotoxin are summarized by the data compiled in Table 1.

In nine experiments, a dose of 1 μg/kg reduced blood pressure to an average level of 99 ± 6/63 ± 5 mm Hg, although in two of these this dose was completely without effect. The heart rate fell in three of the seven experiments in which the toxin had a hypotensive effect, and the greatest slowing amounted to 20%. At doses of 2.5 to 10 μg/kg, the toxin always produced a substantial fall in systolic and diastolic blood pressures, pulse pressure, and heart rate. The maximum reduction of blood pressure occurred at a dose of 5 μg/kg; bradycardia was maximal at a dose of 2.5 μg/kg. When the heart rate was recorded continuously with a Grass tachograph, we observed that the bradycardia was concurrent with the fall in arterial pressure (Fig. 1, e, and 5, A).

In two cats, acute sympathetic denervation of the heart by removal of the thoracic sympathetic chains and the stellate ganglia on both sides resulted in a fall in heart rate from 144 to 102 in one animal, and from 240 to 100 in the other. The administration of as much toxin as 15 μg/kg did not further decrease the heart rate but, in fact, increased it to 120 and 111, respectively. We believe that this effect was due to inhibition of conduction in vagal fibers, since in five other animals we were able to demonstrate that 2.5 to 5 μg/kg of toxin would effectively prevent slowing of the heart due to electrical stimulation of the vagus nerve.

The fall in heart rate was a sinus bradycardia, as mainly the P-P interval was increased. There was little or no effect on either atrioventricular conduction time (P-R) or intraventricular conduction (Q-S) (Table 1). However, the lack of effect of toxin on sympathetically denervated hearts, as well as experiments on spontaneously beating isolated right atria, to be described below, indicate that bradycardia is due to blockade of conduction in sympathetic fibers carrying impulses to the pacemaker region and not to a direct effect on cells of the sinoatrial node.
In some experiments, a sudden fall in pulse rate to about one-half normal was seen after injection of toxin (Fig. 1). In those experiments in which ECG was recorded, there was no evidence of an A-V nodal conduction block. Some arterial pulse records, on the other hand, show periods of pulsus alternans (Fig. 1, f) in spite of normal ECG waves. True A-V dissociation was seen occasionally, but only after considerably higher doses of toxin.

EFFECTS OF TETRODOTOXIN ON VENTRICULAR PRESSURE AND FORCE, CARDIAC OUTPUT, AND ADRENAL MEDULLARY STIMULATION

Stimulation of the thoracic sympathetic chains or postganglionic cardiac fibers of the stellate ganglia increased cardiac rate, intraventricular pressure, and arterial pressure in six open-chest cats (Fig. 2, A and B). After injection of toxin, left ventricular and arterial pressure fell, and the responses to sympathetic nerve stimulation were greatly decreased or completely abolished (Fig. 2, A and B).

Blockade of conduction in sympathetic postganglionic nerves by tetrodotoxin was also demonstrated by pharmacologic methods. Intravenous injection of low doses of DMPP (1,1-dimethyl-4-phenyl-piperazinium iodide, 5 to 20 μg/kg) increased blood pressure and heart rate by stimulation of the adrenal medulla and sympathetic ganglia. After bilateral adrenalectomy (two cats) higher doses of DMPP (40 to 100 μg/kg) were required to elicit a comparable hypertensive effect. This effect of DMPP was markedly inhibited by tetrodotoxin (Fig. 2, C).
Effect of tetrodotoxin on arterial blood pressure (ART) and left ventricular force (LVF). (A) 2-kg cat. Effect of stimulation of left thoracic sympathetic chain (5 v, 10/sec), at S. After intravenous injection of toxin, ventricular force was reduced and response to nerve stimulation was almost abolished (B). Response to norepinephrine (NE) unaffected (C).

probably by blockade of conduction of impulses in the sympathetic postganglionic fibers. The persistence of some increase in blood pressure and heart rate following high doses of DMPP after tetrodotoxin, in adrenalectomized animals, is most likely the result of direct release of endogenous tissue catecholamines from nerve terminals by DMPP, especially within the heart (19-21).

After intravenous injection of 2.5 μg/kg tetrodotoxin, the force of ventricular contraction decreased by 54 ± 4.5% in eight experiments (6 left ventricles, 2 right ventricles); the velocity of contraction was also decreased (Fig. 3). Prior to administration of the toxin, stimulation of the pre- or postganglionic fibers of the stellate ganglia in these animals increased ventricular force by 50% to 76%; however, after the administration of the toxin this positive inotropic effect was abolished (Fig. 3, A and B). On the other hand, the response to norepinephrine was not impaired (Fig. 3, C). If the arterial pressure was maintained in these same animals at a low level, similar to that after toxin, by the infusion of histamine or acetylcholine, ventricular force actually increased during the infusion of histamine and fell by an average of only 10% with acetylcholine.

The effect of tetrodotoxin on the cardiac output is shown in Table 2. In seven experiments, 2.5 μg/kg toxin administered intravenously reduced the cardiac output by an average of 50%, and the stroke volume by 35%. Thus the decrease in cardiac output is due not only to a decreased stroke volume, but also to the attendant bradycardia. In four open-chest animals, a Henderson cardiometer (22) placed around the ventricles was used to measure beat-to-beat variations in ventricular volume. These experiments confirmed that stroke volume was decreased after administration of tetrodotoxin.

### TABLE 2

**Effect of Tetrodotoxin on Cardiac Output**

<table>
<thead>
<tr>
<th>No. experiments</th>
<th>Arterial blood pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Cardiac output (liters/min)</th>
<th>Calculated stroke volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Controls</td>
<td>163 ± 11/113 ± 10</td>
<td>176 ± 15</td>
<td>.346 ± .030</td>
</tr>
<tr>
<td>7</td>
<td>After toxin (2.5 μg/kg)</td>
<td>74 ± 6/42 ± 4</td>
<td>145 ± 14</td>
<td>.176 ± .028</td>
</tr>
<tr>
<td>5</td>
<td>After recovery from toxin</td>
<td>166 ± 14/114 ± 14</td>
<td>162 ± 19</td>
<td>.304 ± .043</td>
</tr>
</tbody>
</table>

All values are mean ± SEM.
CARDIOVASCULAR ACTIONS OF TETRODOTOXIN

Another important effect of the toxin was shown in four cats in which 2.5 to 5 µg/kg tetrodotoxin blocked the hypertensive response to electrical stimulation of fibers from the celiac ganglia or splanchnic nerve innervating the adrenal medulla. Tetrodotoxin does not prevent direct chemical stimulation of the adrenal medulla by acetylcholine, nicotine, or DMPP (1).

EFFECTS OF TETRODOTOXIN ON VASCULAR RESISTANCE IN PERFUSED HINDLIMBS

Tetrodotoxin, injected intravenously in three cats, produced a fall in the perfusion pressure of the hindlimbs concurrently with the fall in arterial pressure and heart rate (Fig. 4, A). Thus the hypotension is due in large measure to peripheral vasodilation. To determine if the vasodilation was the result of sympathetic vasomotor nerve block, the effects of the toxin on the pressor response to electrical stimulation of the lumbar sympathetic chain and the postganglionic sympathetic fibers running in the sciatic nerve trunk were studied in ten cats. To prevent the marked vasodilation resulting from contraction of leg muscles due to sciatic nerve stimulation, d-tubocurarine, 0.3 to 1.0 mg/kg iv, was administered. The pressor responses to electrical stimulation of either pre- or postganglionic vasomotor nerves were inhibited equally by intravenous or intraarterial injections of the toxin (Fig. 4, D). Intraarterial injection of 0.5 µg/kg toxin decreased the maximal vasoconstrictor response by about 30%, whereas 1.2 µg/kg produced about 85% inhibition. The vasoconstrictor action of norepinephrine was not altered.

The possibility of a direct action of tetrodotoxin on vascular smooth muscle as suggested by Lipsius and co-workers (6), or a
Peripheral vascular actions of tetrodotoxin. (A) Responses of perfused hindlimbs in 2.3-kg cat to intraarterial injection of norepinephrine (NE), epinephrine (E), acetylcholine (Ach), histamine (H), and tetrodotoxin (TOX). After maximal vasodilation with toxin, histamine produced a further fall in perfusion pressure (PP). (B) After administration of 10 mg of phenoxybenzamine to the same cat. No additional vasodilation produced by twice the original dose of toxin. Histamine and acetylcholine remained effective, and the original pressor effect of epinephrine was reversed. Initial sharp rise and slight fall in pressure records after injections are injection artifacts. (C) 2-kg cat. Effect of toxin and guanethidine on response to lumbar sympathetic chain stimulation at S (5 v, 10/sec). (D) Fall in perfusion pressure of hindlimbs after tying ligatures placed around both adrenals. Initial brief fall in perfusion pressure after toxin is an injection artifact, but note marked vasodilation due to isoproterenol (I). (E) 2-kg cat. Response to stimulation of lumbar sympathetic chain at S. After recovery from toxin effects, bilateral adrenalectomy and sympathetic denervation of the hindlimbs resulted in a fall in perfusion pressure. Large dose of toxin had no greater effect than injection of same volume of saline (Sal), but other vasodilators remained effective.

histamine-like action, as suggested by Li (4) was investigated. We assumed that any direct vasodilator action on vascular smooth muscle should be apparent even after complete blockade of transmitter release from vasoconstrictor nerves or α-receptor blockade. The effect of tetrodotoxin on the circulation in the perfused hindlimbs was therefore studied after treatment with guanethidine or β-TM 10⁴ to abolish the release of norepinephrine when adrenergic nerves were stimulated, or sufficient phenoxybenzamine to abolish the vasoconstrictor response to norepinephrine. In 23
CARDIOVASCULAR ACTIONS OF TETRODOTOXIN

A

\begin{align*}
\text{POST} & \quad \text{PRE} \\
0' & \quad 0' \\
10' & \quad 2 \times 10^{-8} \\
15' & \quad \text{w} \\
17' & \quad \text{w} \\
80' & \quad \text{w}
\end{align*}

\text{FIGURE 6}

Isolated cat right atrium, at 30°C, stimulated at 1/sec, 5 v. Supramaximal electrical stimulation (50 v) or addition of tyramine (tyr) to the bath produced a positive inotropic response. Response to supramaximal electrical stimulation was reversibly abolished by toxin but not the response to tyramine. (B) Isolated cat right atrium with sympathetic nerves and stellate ganglion intact, at 30°C, contracting spontaneously. At S alternate stimulation of pre- and postganglionic nerve trunks was maintained for the period indicated by the horizontal lines. Tetrodotoxin inhibited both responses to about the same extent. This effect was slowly reversible upon washing (w) the toxin out of the bath.

out of 30 experiments, tetrodotoxin had no additional vasodilator action after such treatment (Fig. 5, A and B). In five experiments, phenoxybenzamine, 10 mg/kg, did not completely abolish the pressor response to injected norepinephrine. An additional dose of phenoxybenzamine in each case effectively abolished the responses to both norepinephrine and tetrodotoxin. Under these conditions, vasodilation was still consistently produced by histamine, acetylcholine, isoproterenol, and epinephrine.

In two experiments, the initial fall in perfusion pressure due to \( \beta \)-TM 10 or guanethidine was followed by a gradual rise toward normal for 30 to 60 minutes, after which the injection of tetrodotoxin then produced a further fall in perfusion pressure. We thought that the tendency of the blood pressure to rise after the maximal vasodilation produced by \( \beta \)-TM 10 or guanethidine might be due to increased, reflexly induced, adrenal medullary secretion. Since the toxin had previously blocked neural stimulation of the adrenal medulla, the fall in hindlimb perfusion pressure could have resulted from such an action, which led to a decrease in circulating catecholamines. This interpretation appears
to be correct, since acute bilateral adrenalectomy performed in four cats 30 minutes after administration of $\beta$-TM 10 or guanethidine resulted in a prompt fall in hindlimb perfusion pressure, ranging from 18 to 35 mm Hg (Fig. 5, D). Thereafter, injections of tetrodotoxin in doses as high as 10 $\mu$g/kg into the hindlimb circulation were without effect. Histamine, isoproterenol, and acetylcholine, however, always produced additional vasodilation with falls in perfusion pressure of 30 to 45 mm Hg. In two cats, acute sympathetic denervation of the hindlimbs (lumbar sympathetic chains removed and sciatic nerve trunks sectioned) accompanied by bilateral adrenalectomy also resulted in a fall in perfusion pressure, after which tetrodotoxin was without further effect (Fig. 5, E). Under these conditions, the direct vasodilators referred to above still produced additional vasodilation.

In three intact cats, after several doses of 2.5 to 5 $\mu$g/kg of toxin, or a single dose of 10 $\mu$g/kg, a state was obtained in which there was prolonged, severe hypotension and abolition of sympathetic vasoconstrictor nerve responses. Under these conditions, repeated additional doses of toxin no longer affected the arterial blood pressure. Yet histamine, isoproterenol, and acetylcholine consistently and repeatedly lowered the blood pressure even further. A similar observation was made by Koizumi and her co-workers (5) using acetylcholine. They also were unable to prevent the hypotensive action of the toxin with an antihistaminic compound.

**EFFECTS OF TETRODOTOXIN ON ISOLATED RIGHT ATRIUM AND SYMPATHETIC NERVE-ATRIUM PREPARATIONS**

Stimulation of the intrinsic sympathetic nerve endings in the isolated right atrium by supramaximal voltage markedly increases the force of contraction of electrically driven muscles (23). This effect was completely abolished in five experiments by adding as little as 1 to $2 \times 10^{-8}$ g/ml toxin to the tissue bath without affecting the response to the direct action of catecholamines or the catecholamine-releasing action of tyramine (Fig. 6, A). Thus the toxin probably acts by inhibiting the conduction of impulses in nerve fibers without affecting the mechanism for release of the neurotransmitter. This interpretation is consistent with the recent experiments of Katz and Miledi (24), who showed that tetrodotoxin, while blocking action potential conduction in motor nerves, did not hinder the release of acetylcholine due to local depolarization of the nerve terminals.

In five atria isolated with their sympathetic nerves intact, the stimulation of either pre- or postganglionic fibers increased the force and spontaneous frequency of contractions (Fig. 6, B). As little as $5 \times 10^{-9}$ g/ml toxin partially inhibited these responses to nerve stimulation, and complete block was attained at 1 to $2 \times 10^{-8}$ g/ml. Responses to pre- and postganglionic stimulation were reduced equally.

Assuming that after a single intravenous injection into an intact cat, tetrodotoxin is distributed mainly in the extracellular fluid during the first few minutes required to attain maximal pharmacologic effect, one can calculate an approximation of the possible toxin content of the extracellular water. These calculations give figures of $5 \times 10^{-9}$ to $2.5 \times 10^{-8}$ g/ml after doses of 1 to 5 $\mu$g/kg, which is in the range found to block sympathetic nerves in vitro.

**FIGURE 7**

Effect of high doses of tetrodotoxin on spontaneous contractions (upper trace) and action potentials (suction electrode) (lower trace) in isolated cat right atrium. Temperature 35°C. (A) Control. (B) After addition of $2 \times 10^{-7}$ g/ml norepinephrine. (C) After washout of norepinephrine and 5 minutes after addition of $2 \times 10^{-4}$ g/ml tetrodotoxin. (D) 10 minutes after addition of toxin. (E) Addition of $2 \times 10^{-7}$ g/ml norepinephrine in the presence of toxin. (F) 10 minutes after washout of both norepinephrine and toxin.

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CARDIOVASCULAR ACTIONS OF TETRODOTOXIN

A 100'

FIGURE 8

(A) Hypotensive effect of tetrodotoxin, injected intravenously in a spinal cat. Femoral arterial pressure in a 1.8-kg cat.

(B) Femoral arterial pressure in a 2-kg cat. At S the sigmoid gyrus of the cerebral cortex on the left side was stimulated electrically with a bipolar electrode (10 V, 20/sec for 15 seconds). The pressor response is markedly inhibited after the intravenous injection of tetrodotoxin. After 20 minutes the blood pressure returned to normal and stimulation of the cortex evoked a pressor response equal to that of the control.

At the time that the responses to sympathetic nerve stimulation were abolished, neither the spontaneous rate nor force of contraction of the atria was altered. In one experiment, a slight reduction in force was noted at a toxin concentration of $5 \times 10^{-7}$ g/ml. Higher concentrations caused a slowing of rate, arrhythmias, and further reduction of force, until at 1 to $2 \times 10^{-6}$ g/ml, contractions were abolished in the five atria tested. The atrial action potential was also abolished at these concentrations, but spontaneous electrical and mechanical activity could be restored by applying norepinephrine (Fig. 7).

EFFECTS OF TETRODOTOXIN IN SPINAL CATS AND ON A CEREBRAL VASOMOTOR CENTER

Two observations tend to support the contention of Kao and his co-workers (9) that the hypotensive action of the toxin does not result from a selective action on the brain. First, we found that 2.5 \( \mu \)g/kg toxin lowered blood pressure in five spinal cats (Fig. 8, A) from 94/48 mm Hg (range: 105/55 to 75/40) to 65/28 mm Hg (range: 75/35 to 50/20). This is almost precisely the same level to which the arterial pressure was lowered in normal animals at this dose of toxin. Of course, the total magnitude of the pressure drop is less in spinal animals because of the preexistent low pressure resulting from the removal of vasomotor influence of the brain.

Second, in two animals, electrical stimulation of the vasomotor area of the sigmoid gyrus of the cerebral cortex increased arterial pressure (Fig. 8, B). This effect has been shown to be accompanied by increased frequency of action potential discharge in sympathetic nerves to the heart (25). The dose of toxin required to abolish this response to cerebral stimulation was the same as that which blocked the effects of peripheral sympathetic neuron stimulation, namely 2.5 to 5 \( \mu \)g/kg. Responses to brain stimulation were not affected by doses which were too low to reduce the effects of peripheral sympathetic nerve stimulation.

Discussion

We conclude from the results described above that the hypotensive action of tetrodotoxin in the cat is the result of a combination of peripheral vasodilation and decreased cardiac output. The latter is a consequence of a decrease in both heart rate and stroke volume. These effects of tetrodotoxin on the peripheral circulation and the heart coincide in time and intensity with an inhibition of responses to stimulation of postganglionic sympathetic nerve fibers innervating blood vessels and the heart, as well as splanchnic nerve fibers innervating the adrenal medulla. The recovery of the blood pressure to normal is associated with a return of these nerves to their normal responsiveness.

It should be pointed out that in the dose range producing hypotension, tetrodotoxin has also been found to inhibit motor nerves to skeletal muscle, some afferent nerves, and certain areas in the central nervous system (1, 5). Thus it is found that muscular weakness, respiratory depression, and hypotension are always concurrent (1).

The toxin did not block responses to stimulation of preganglionic sympathetic nerves.
without a comparable effect on responses to postganglionic nerve stimulation. Nor did it interfere with adrenergic transmitter release by such agents as tyramine, or the direct action of the catecholamines on their target tissues. Thus the action of tetrodotoxin, which is due to the blockade of conduction in axons, is clearly distinguishable pharmacologically from adrenergic receptor-blocking agents such as phenoxybenzamine or agents which block transmitter release from adrenergic nerve terminals, such as β-TM 10 and guanethidine. Peripheral vasodilation in the cat is the result of abolition of sympathetic vasoconstrictor tone and not to a direct action on vascular smooth muscle, because it did not occur after effective α-receptor blockade, or after adrenalectomy accompanied by blockade of adrenergic transmitter release or denervation. The inability to demonstrate a direct vasodilating action of tetrodotoxin on vascular smooth muscle in the cat was not entirely unexpected. The toxin has been shown to have no effect on the tone of isolated arterial strips, although it abolished responses to vasoconstrictor nerve stimulation (26) and it also failed to block action potentials in arterial (27) and venous (28) smooth muscle. A number of other isolated smooth muscle preparations have also been shown to be unaffected by tetrodotoxin (29, 30). Whether the reports of direct vasodilating action in dog (6) and rat (4) can be attributed to differences in the vascular muscle of these species is not known at present.

Our observations indicate that the hypotensive action of the toxin in the cat cannot be ascribed to a selective action on the brain vasomotor centers. The cardiovascular depression induced by the toxin was consistently elicited in spinal animals, and the inhibition of responses to stimulation of a brain vasomotor center was apparent only at doses of the drug which produced peripheral sympathetic neuron blockade. Recent experiments in other laboratories concerning the central actions of tetrodotoxin are contradictory. Cross-perfusion experiments in cats and dogs (9) failed to show any hypotensive action of the toxin when it circulated through the recipient animal’s head only. However, the doses may not have been sufficient, since they were calculated on the basis of brain weight rather than the proportion of the total cardiac output which goes to the brain. On the other hand, Koizumi and co-workers (5) clearly demonstrated that doses of toxin in the range of 1 to 4 μg/kg produced partial to complete block of mono- and polysynaptic spinal reflexes, as well as cortically evoked responses in cats. The significance of these findings vis-à-vis the circulatory effects of the toxin is difficult to assess for two reasons. First, neither the spinal reflexes nor the cortical areas studied were shown to be involved in vasomotor regulation; and second, no temporal correspondence between the spinal and cortical effects and the hypotension was found. The latter investigators attribute the hypotension primarily to action of the toxin on the medullary vasomotor centers, because it presumably did not occur in spinal animals. Both we and Murtha and co-workers (3) have found that the toxin acts on spinal animals, although quantitatively the absolute magnitude of the fall in pressure must of necessity be less than in intact animals because of the preexistent loss of vasomotor influences of the brain. One experiment described by Koizumi and her co-workers (5) does, in fact, show this. Tetrodotoxin, 2 μg/kg, did reduce blood pressure in a spinal cat to precisely the same level to which it had previously reduced it in the same animal prior to spinal section. We would conclude that, although the toxin may in fact actually depress central nervous system vasomotor centers, no selective action—that is, without concurrent blockade of peripheral cardiac and vasomotor nerves—on such areas has yet been convincingly demonstrated. The peripheral nerve-blocking effects of tetrodotoxin which have been demonstrated correspond in time and intensity to the observed depression of the circulation and are therefore entirely sufficient to account for the principal pharmacologic actions of the toxin on the cardiovascular system.
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