MOLECULAR PHARMACOLOGY, 20:526–532



# High-Affinity Saxitoxin Receptor Sites in Vertebrate Heart

## Evidence for Sites Associated with Autonomic Nerve Endings

WILLIAM A. CATTERALL AND JEFFREY COPPERSMITH

*Department of Pharmacology, University of Washington, Seattle, Washington 98195*

Received June 9, 1981; Accepted August 10, 1981

SUMMARY

We have studied the binding of [$^3$H]saxitoxin (STX) to total particulate preparations from ventricles of vertebrate heart. In each of the six species studied, a single class of high-affinity receptor sites was detected with $B_{max}$ values of 1.7 fmoles/mg (wet weight) to 12.0 fmoles/mg (wet weight) and $K_D$ values of 0.3 nM–7.5 nM for STX and 3.5 nM–10.4 nM for tetrodotoxin (TTX). In the bullfrog heart, two classes of high-affinity STX receptor sites were observed. One class of sites (28%) had high affinity for TTX and resembled STX/TTX sites in bullfrog brain. A second class (72%) had very low affinity for TTX and were apparently specific to the heart. In chick heart, the high-affinity STX/TTX receptor sites had properties similar to myocardial sodium channels studied physiologically. In contrast, for mammalian hearts, the $K_D$ of high-affinity STX/TTX receptor sites for TTX (3.5 nM–10 nM) does not correlate with the inhibition of myocardial sodium channels by high concentrations of TTX (800 nM–3000 nM) in physiological experiments. In the rat, treatment with 6-hydroxydopamine caused a loss of 50–60% of the high-affinity STX/TTX receptor sites in the ventricles, consistent with the conclusion that a major fraction of these high-affinity toxin binding sites is associated with autonomic nerve endings in the rat. Taken together, the results show that vertebrate hearts contain a substantial complement of high-affinity STX/TTX receptor sites that are associated with autonomic nerve endings. In mammalian heart, there may be no high-affinity STX/TTX receptor sites associated with myocardial sodium channels.

### INTRODUCTION

Voltage-sensitive sodium channels are responsible for the initial rapid upstroke (phase 0) of the cardiac action potential (reviewed in ref. 1). These sodium channels are similar to those in nerve and skeletal muscle in many respects. The voltage and time dependence of activation and inactivation can be fit by a model similar to that derived by Hodgkin and Huxley (2) for sodium channels in nerve (1, 3–5). Sodium channels in heart are also inhibited by tetrodotoxin and saxitoxin (1), specific blockers of sodium channels in nerve and skeletal muscle (reviewed in refs. 1 and 6). In most species studied, relatively high concentrations of tetrodotoxin and saxitoxin are required for inhibition (7–18).

[$^3$H]STX[1] and [$^3$H]TTX have proven to be valuable tools in determining the density and location of sodium channels in nerve and muscle by radioligand binding methods (reviewed in refs. 1 and 19). More recently, these labeled toxins have also been used in identification and purification of protein components of sodium channels from electric eel (20), rat seletal muscle (21), and rat brain (22, 23). It would be of considerable interest to identify and purify sodium channel components from rat heart and to compare the molecular properties of sodium channels in heart, brain, and skeletal muscle. In the experiments presented here, we have studied the binding of [$^3$H]STX to particulate preparations from hearts of rats and other vertebrates to characteriz the STX receptor sites in vertebrate heart and to determine whether STX binding to cardiac sodium channels can be used in further studies of the molecular properties of these sodium channels. While this work was being prepared for publication, Lombet *et al.* (24) described binding of a tetrodotoxin derivative to rat heart. Their results are considered under Discussion.

### EXPERIMENTAL PROCEDURES

*Materials.* Saxitoxin was obtained from the National Institutes of Health, Bethesda, Md. It was radioactively labeled by the specific $^3H_2O$ exchange method of Ritchie *et al.* (25) and purified and characterized as described previously (26). The preparations used ranged from 75% to 85% radiochemical purity.

*Measurements of [$^3$H]STX binding.* Hearts were dissected, cut open, and rinsed to remove blood with standard binding medium consisting of 130 mM choline chloride, 5.4 mM KCl, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (adjusted to pH 7.4 with Tris

[1] The abbreviations used are: STX, saxitoxin; TTX, tetrodotoxin.

0026-895X/81/030526-07$02.00/0

base), 0.8 mM $MgSO_4$, and 5.5 mM glucose. The hearts were then weighed and homogenized with a Teflon/glass homogenizer at 10% (w/v) in ice-cold standard binding medium. The membrane fraction was sedimented at 120,000 × *g* for 20 min, resuspended in the same volume of standard binding medium, and filtered through a layer of nylon mesh (Nitex, 150 μm diameter) to remove blood vessels and clumps of tissue. This total particulate preparation was then used in [$^3$H]STX binding assays.

Unless otherwise specified, binding of [$^3$H]STX was initiated by mixing 200 μl of the heart membrane homogenate with 50 μl of standard binding medium containing [$^3$H]STX and the other effectors noted in the figure legends as described in previous work with brain membranes (26). Samples were incubated for 20 min at 0°. The binding reaction was terminated by dilution of the reaction medium with 3 ml of ice-cold wash medium consisting of 163 mM choline chloride, 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (adjusted to pH 7.4 with Tris base), 1.8 mM $CaCl_2$, and 0.8 mM $MgSO_4$. Bound [$^3$H]STX was then measured by collection of the membranes on glass-fiber filters (Whatman GF/C) and washing three times with additional 3-ml volumes of ice-cold wash medium. The filters were placed in counting vials, and counts per minute of bound [$^3$H]STX were measured by liquid scintillation counting. Nonspecific binding was measured in parallel incubations in the presence of 1 μM or 10 μM TTX. When compared, nonspecific binding values were identical at these two concentrations of TTX.

*Treatment with 6-hydroxydopamine.* Male Sprague-Dawley rats (200–250 g) received i.p. injections of 6-hydroxydopamine in a vehicle of 0.1% (w/v) sodium ascorbate and 0.9% (w/v) NaCl. Control animals received injections of vehicle only. Treatment regimens are described in the individual figures.

## RESULTS

*Binding of [$^3$H]STX to sodium channels in rat heart.* The properties of STX receptor sites in rat heart were examined using total particulate preparations from rat ventricle and a filtration assay technique as described under Experimental Procedures. Specific binding to sodium channels was determined by subtracting nonspecific binding measured in the presence of 10 μM TTX from total binding. For rat heart, nonspecific binding was less than 10% of total binding. The time dependence of specific [$^3$H]STX binding is illustrated in Fig. 1A. The membrane homogenate was incubated with 3 nM [$^3$H]STX at 0° for the indicated times, and bound [$^3$H]STX was determined by filtration. Specific binding of [$^3$H]STX was complete within 5 min of incubation under these conditions. For subsequent experiments, an incubation time of 20 min was chosen.

The half-time for dissociation of specifically bound STX was determined using the rapid filtration assay. The membrane homogenate was incubated for 20 min at 0° with 3 nM [$^3$H]STX and then TTX was added to a final concentration of 10 μM to block further binding. The [$^3$H]STX remaining bound at increasing times was then measured by rapid filtration and washing within 10-sec. The STX-receptor complex dissociated with a half-time of 4 min (Fig. 1B). Therefore, the dissociation occurring during the wash period (10 sec) can be ignored.

The concentration dependence of [$^3$H]STX binding is illustrated in Fig. 2. With increasing concentrations of [$^3$H]STX, total binding (○) increases to a plateau value (Fig. 2A.) Nonspecific binding (●) varied linearly with [$^3$H]STX concentration. At 3 nM [$^3$H]STX, more than 95% of [$^3$H]STX binding is specific. In Fig. 2B, the specific component of binding is presented as a Scatchard plot. A single class of sites with a $K_D$ of 2.5 nM and $B_{max}$

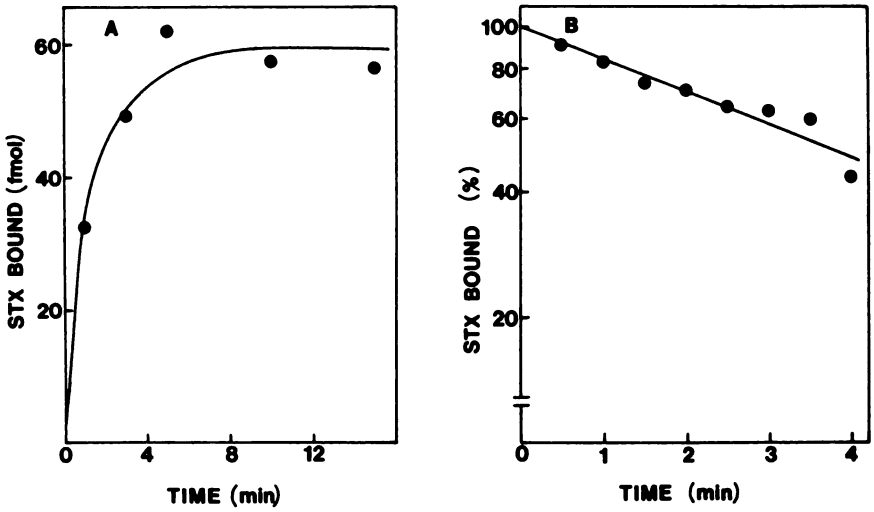


FIG. 1. *Time course of formation and dissociation of the saxitoxin/receptor complex*

A. Membrane homogenate from rat heart was incubated with 3 nM [$^3$H]STX at 0° for the time indicated, and specifically bound [$^3$H]STX was determined as described under Experimental Procedures.

B. Membrane homogenate was incubated with 3 nM [$^3$H]STX for 20 min at 0°. TTX was added to a concentration of 10 μM, and specifically bound [$^3$H]STX was measured at the indicated times after addition of TTX as described under Experimental Procedures.

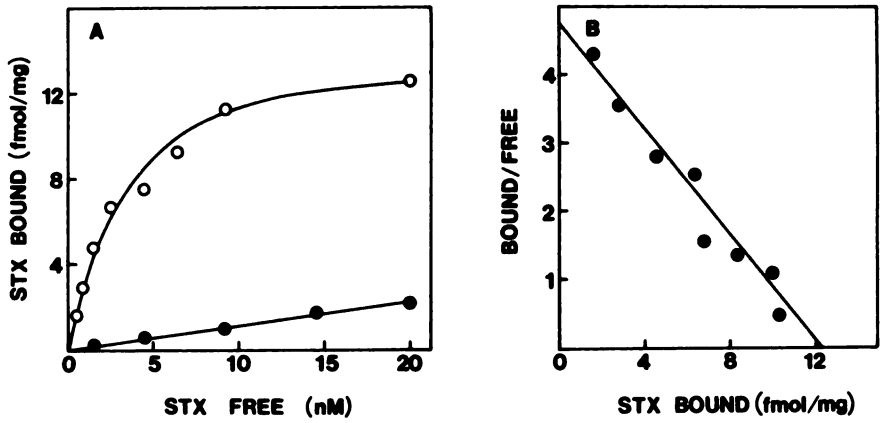


FIG. 2. *Scatchard analysis of [$^3$H]STX binding*

A. Membrane homogenate from rat heart was incubated for 20 min at 0° with increasing concentrations of [$^3$H]STX alone (○) or in the presence of 10 μM unlabeled TTX (●), and bound [$^3$H]STX was determined as described under Experimental Procedures.

B. The specific binding data of A are presented as a Scatchard plot where the ordinate is femtomoles per milligram per nanomolar and the abscissa is femtomoles per milligram.

of 12.1 fmoles/mg (wet weight) is observed over the concentration range tested. In 14 separate experiments, the mean values were $K_D$ = 2.1 ± 1.0 nM and $B_{max}$ = 12.0 ± 1.2 fmoles/mg.

The $K_D$ for TTX binding to these STX receptor sites was measured in competitive displacement experiments. One-half of the binding of 3 nM [$^3$H]STX was displaced by 3.5 nM TTX, indicating a $K_D$ of 1.9 nM for TTX binding at 0°. Similar xperiments carried out at 36° gave values of 9.1 nM for the $K_d$ for STX and 10.0 nM for TTX (data not shown). These binding data show that rat ventricles contain a significant number of high-affinity binding sites for TTX and STX with $K_D$ values for these toxins that are similar to those of sodium channels in nerve and skeletal muscle.

*Comparison of STX and TTX binding and inhibition of sodium channels in hearts of different species.* Inhibition of sodium channels in heart by TTX has been investigated in several species using either electrophysiological methods or ion flux methods. These data are summarized in Table 1. Concentration-effect curves have been derived from measurements of the rate of rise of the action potential ($dV/dT$) for chick and guinea pig (7, 12), from voltage-clamp data for rabbit (11), and from ion flux data on cultured cells for rat and chick (13–16, 18). In addition, the effects of a few concentrations of TTX on electrophysiological parameters have been determined in dog, frog, sheep, and calf preparations (8–10, 17). In all mammals studied, $K_{0.5}$ for TTX inhibition is 1 μM or greater. In contrast, in chick $K_{0.5}$ values range from 3 nM to 20 nM in several studies, and less-complete investigations on the European grass frog *Rana esculenta* indicate a $K_{0.5}$ of substantially less than 100 nM (17). These electrophysiological and ion flux data suggest that nonmammalian vertebrates might have myocardial sodium channels with high affinity for TTX but mammals should not.

To determine whether there is any correlation between the $K_D$ for high-affinity STX and TTX receptor sites and the $K_{0.5}$ for block of myocardial sodium channels by STX and TTX, we examined [$^3$H]STX binding to total particulate preparations from hearts of various mammalian and nonmammalian vertebrates. In each case, the membrane homogenate was prepared as described for rat heart under Experimental Procedures, and the experiments to determine $B_{max}$ and $K_D$ for STX and $K_D$ for

TABLE 1

*Inhibition of sodium channels in hearts of different species by tetrodotoxin*

| Species | $K_{0.5}$ | Method | Reference |
|---|---|---|---|
| | *nM* | | |
| Rat | 1000 | Ion flux | 18 |
| Guinea pig | 2000 | *dV/dt* | 7 |
| Dog | 3000 | *dV/dt* | 8 |
| Cow | >3300 | Voltage clamp | 10 |
| Sheep | >3300 | Voltage clamp | 9, 1 |
| Rabbit | 820 | Voltage clamp | 11 |
| Chicken | 20 | *dV/dt* | 12 |
| | 3–10 | Ion flux | 13–16 |
| Frog (*Rana esculenta*) | <50 | *dV/dt* | 17 |

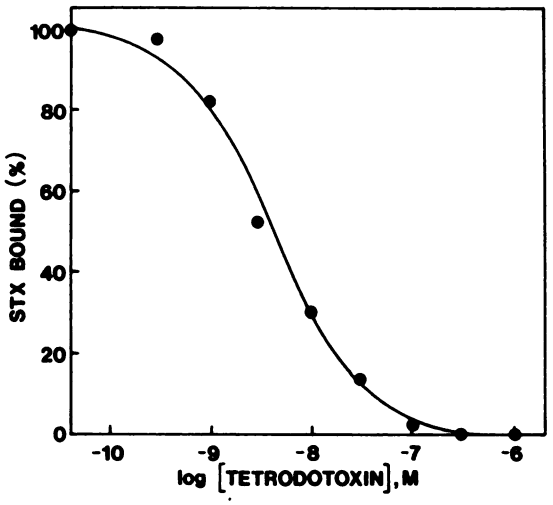


FIG. 3. *Competitive displacement of [$^3$H]STX by TTX*

Membrane homogenate from rat heart was incubated with 3 nM [$^3$H] STX and the indicated concentrations of TTX for 20 min at 0°, and bound [$^3$H]STX was determined as described under Experimental Procedures.

TTX were carried out as in Figs. 2 and 3. The results show that all six species have a substantial complement of high-affinity STX receptor sites in their ventricles, with $K_D$ values ranging from 0.3 nM to 7.5 nM for STX and from 3.5 nM to 10.4 nM for TTX. The tissue density of binding sites ranged from 1.7 fmoles/mg for bullfrog to 11.8 fmoles/mg for guinea pig. Comparison of the binding data of Table 2 with the physiological data of Table 1 reveals no correlation between the presence of high-affinity STX/TTX receptor sites and the inhibition of sodium channels by TTX. The site densities in mammalian hearts are as high and the $K_D$ values as low as in chick heart, although the values of $K_{0.5}$ for block of sodium channels are 100-fold greater in mammalian heart. These results suggest that the high-affinity STX/TTX sites observed in binding studies are not associated with the myocardial sodium channels studied in the physiological experiments.

The mammalian cardiac muscle preparations studied physiologically have included ventricular muscle from calf and sheep (9, 10), Purkinje fibers from rabbit (11), papillary muscle from guinea pig (7), and dissociated cell cultures from rat (18). Since all of these preparations from different ventricular regions have values of $K_{0.5}$ for TTX that are approximately 1 μM, it is likely that sodium channels in both ventricular muscle and the conduction system have low affinity for TTX. The high-affinity STX/TTX receptor sites that we observe in binding studies must therefore be associated with physiologically inactive sodium channels in cardiac muscle or with autonomic nerve endings present in the ventricles. Evidence in favor of this latter interpretation is presented below.

*TTX binding discriminates two classes of sodium channels in bullfrog heart.* The results of binding experiments with bullfrog (*R. catesbiana*) heart differed in an important way from those with other species. Although bound [$^3$H]STX was displaced with a single $K_D$ of 5 nM by unlabeled STX, only 28% of the specifically bound [$^3$H]STX was displaced by TTX (Fig. 4A). Thus, in bullfrog heart, there are two classes of STX receptor sites. One class (28%) of STX receptor sites has high affinity for both STX ($K_D$ = 5 nM) and TTX ($K_d$ = 3 nM), whereas the second class of sites has only high affinity for STX. Competitive displacement curves for [$^3$H]STX binding to bullfrog brain membranes are illustrated in Fig. 4B. The STX receptor sites in bullfrog brain bind STX and TTX with $K_D$ values of 1.2 nM and 7.2 nM, respectively. Thus, the STX receptors that have low affinity for TTX are unlikely to be associated with nerve endings in the bullfrog heart, since sodium channels in bullfrog nerve bind both toxins with high affinity. It seems likely that these unusual STX receptor sites having very low affinity for TTX are associated with the sodium channels of the ventricular myocardium in bullfrog heart and that the 28% of the STX receptor sites that have high affinity for both STX and TTX are associated with the sodium channels in autonomic nerve endings in bullfrog heart. Thus, the different toxin binding properties of sodium channels in bullfrog nerve and heart allow us to estimate that there are 1.22 pmoles of myocardial sodium channels per milligram (wet weight) and 0.48 pmole of nerve ending sodium channels per milligram (wet weight) in bullfrog ventricle.

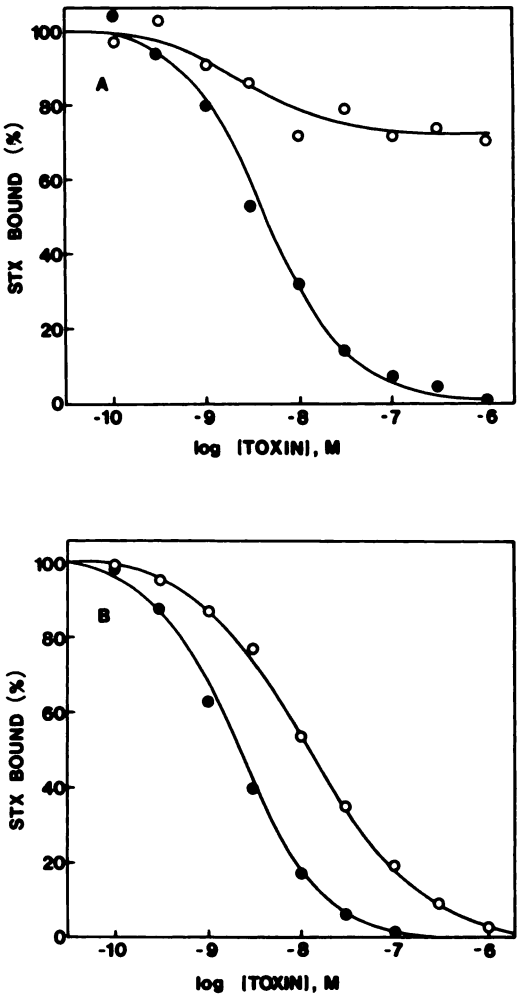


FIG. 4. *Competitive displacement of [$^3$H]STX by unlabeled STX and TTX in bullfrog heart and brain*

Membrane homogenate from bullfrog heart (A) and brain (B) was prepared as described under Experimental Procedures. Samples were incubated for 20 min at 0° with 3 nM [$^3$H]STX and the indicated concentrations of unlabeled STX (●) or TTX (○), and bound [$^3$H]STX was measured as described under Experimental Procedures.

TABLE 2

*Saxitoxin receptors in ventricles from different species*

Membrane homogenates were prepared and [$^3$H]STX binding was measured in cardiac ventricles from different species. $K_D$ and $B_{max}$ for STX were measured as in Fig. 2, and $K_D$ for TTX was measured as in Fig. 3. For rat heart, values are presented ± standard error of the mean of 14 experiments.

| Species | Saxitoxin | | Tetrodotoxin, $K_D$ | No. |
|---|---|---|---|---|
| | $K_D$ | $B_{max}$ | | |
| | *nM* | *fmoles/mg wet wt.* | *nM* | |
| Rat | 2.1 ± 0.3 | 12.0 ± 1.2 | 3.5 | 14 |
| Bullfrog | 1.4 | 1.7 | — | 4 |
| Chicken | 7.5 | 9.3 | 5.1 | 6 |
| Salmon | 1.5 | 11.0 | 5.2 | 2 |
| Guinea pig | 0.9 | 11.8 | 10.4 | 2 |
| Macaque | 0.3 | 4.4 | 6.9 | 2 |

*Effect of 6-hydroxydopamine treatment on STX receptor sites in rat heart.* Field stimulation of intramural

nerves of rat ventricular myocardium produces no cholinergic response (27), indicating that functional innervation of the rat ventricle is almost entirely adrenergic. Adrenergic nerve endings in rat ventricle are very sensitive to chemical sympathectomy by 6-hydroxydopamine (28, 29). Therefore, if the high-affinity STX/TTX receptor sites observed in rat ventricles in binding experiments are associated with sodium channels in nerve endings, treatment with 6-hydroxydopamine should reduce the density of STX receptor sites. Age-matched adult Sprague-Dawley male rats were divided into two groups and received injections of 6-hydroxydopamine or vehicle as described under Experimental Procedures. In our initial experiments, the dosing regimen of Thoenen and Tranzer (28) was used. Animals received two injections of 6-hydroxydopamine, 34 mg/kg, at 6-hr intervals on days 0 and 7 and were killed on days 12–18. Particulate fractions were prepared and binding assays were carried out as described under Experimental Procedures with paired control and treated groups for each determination. The data from a typical pair of treated and control animals are illustrated in Fig. 5 in the form of a Scatchard plot. Treatment with 6-hydroxydopamine reduces $B_{max}$ from 11.4 fmoles/mg to 5 fmoles/mg without significant effect on $K_D$. In several pairs of animals treated in this manner, $B_{max}$ for controls was 12.1 ± 0.3 fmoles/mg and for treated animals was 6.7 ± 0.7 fmoles/mg, or 55% of the control value (Table 3).

Two other treatment regimens were studied. In treatment schedule II (Table 3), animals were treated with 6-hydroxydopamine, 100 mg/kg, on days 0, 1, 7, and 8 and were killed on days 12–18 for study. With this more rigorous treatment, there was only a slightly greater reduction in $B_{max}$ from 12.0 ± 1.2 fmoles/mg to 5.8 ± 0.6 fmoles/mg, or 48% of control. Finally, the effect of a single 100-mg/kg dose of 6-hydroxydopamine was studied. Animals killed 2–6 days after a single dose of 6-hydroxydopamine had an average of 41% of the STX receptor site density of control littermates (Table 3). These data show that chemical sympathectomy with 6-hydroxydopamine leads to a substantial loss of high-affinity STX receptors from rat ventricles, consistent with the conclusion that a major fraction of the high-affinity STX receptors in the ventricle are associated with autonomic nerve endings.

The time course of the effect of 6-hydroxydopamine is illustrated in Fig. 6. In this experiment, animals received injections of 6-hydroxydopamine, 100 mg/kg, or vehicle on day 0. Paired groups of treated and control animals were killed on days 2, 4, 7, and 9, and [$^3$H]STX binding was measured as described under Experimental Procedures. The average $B_{max}$ of the control animals was 12.3 ± 0.8 fmoles/mg. For treated animals, $B_{max}$ was reduced to 6.4 ± 0.7 fmoles/mg on day 2, remained at that level through day 7, and increased thereafter. Under similar conditions, norepinephrine levels in the ventricle are reduced to 5% by day 1 and return to 45% of control by

TABLE 3

*Effect of 6-hydroxydopamine on saxitoxin receptors in rat heart*

Rats were treated with 6-hydroxydopamine according to the treatment schedules indicated, and $K_D$ and $B_{max}$ for specific [$^3$H]STX binding were measured as in Fig. 2. Values presented are mean ± standard error of the mean for four to seven animals.

| Treatment schedule | Control | | Treated | | |
|---|---|---|---|---|---|
| | $K_D$ | $B_{max}$ | $K_D$ | $B_{max}$ | % |
| | *nM* | *fmoles/mg* | *nM* | *fmoles/mg* | |
| I[a] | 1.3 ± 0.2 | 12.1 ± 0.3 | 0.9 ± 0.1 | 6.7 ± 0.7 | 55 |
| II[b] | 1.1 ± 0.2 | 12.0 ± 1.2 | 0.9 ± 0.2 | 5.8 ± 0.6 | 48 |
| III[c] | 3.2 ± 0.3 | 11.4 ± 0.3 | 3.5 ± 0.5 | 4.7 ± 0.8 | 41 |

[a] Animals received 34 mg/kg twice at 6-hr intervals on days 0 and 7 and were killed on days 12–18.

[b] Animals received 100 mg/kg on days 0, 1, 7, and 8 and were killed on days 12–18.

[c] Animals received 100 mg/kg on day 0 and were killed on days 2–7.

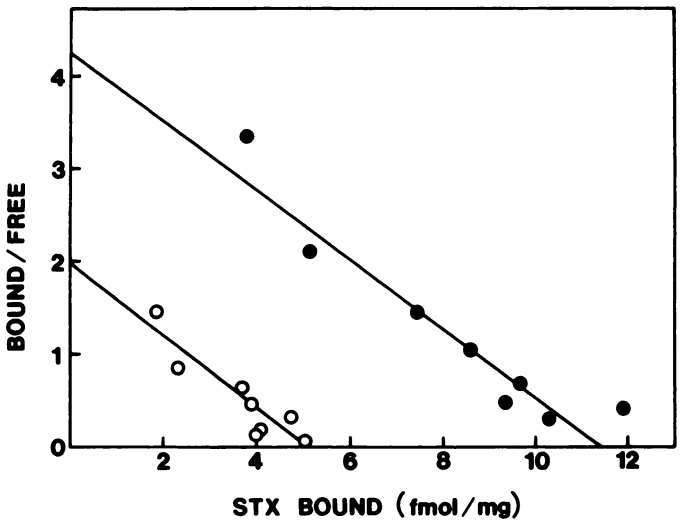


FIG. 5. *Effect of 6-hydroxydopamine treatment on [$^3$H]STX binding in rat heart*

Adult male Sprague-Dawley rats received two injections of 6-hydroxydopmaine, 34 mg/kg, at 6-hr intervals on days 0 and 7 and were killed on day 12. Specific binding of [$^3$H]STX at increasing concentrations was measured as described in Fig. 2 and under Experimental Procedures for treated rats (○) and control rats receiving injections of vehicle only (●). The results are presented as a Scatchard plot.

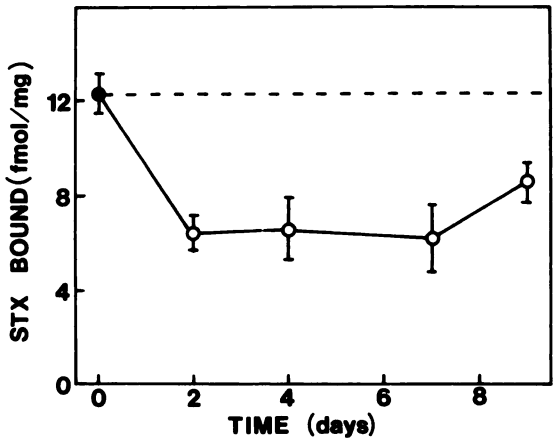


FIG. 6. *Time course of the effect of 6-hydroxydopamine*

Adult male Sprague-Dawley rats received one injection of 6-hydroxydopamine, 100 mg/kg, on day 0. Animals were killed on the indicated days, and specific [$^3$H]STX binding was measured at a saturating concentration (15 nM) of [$^3$H]STX as described under Experimental Procedures.

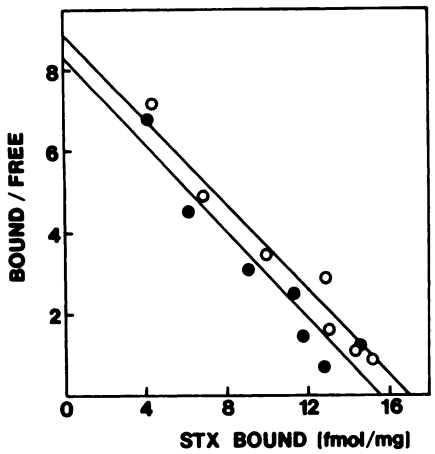


FIG. 7. *Effect of reserpine treatment on [$^3$H]STX binding in rat heart*

Adult male Sprague-Dawley rats received injections of reserpine, 4 mg/kg, on days 0, 2, 4, and 6 and were killed on day 7. Specific binding of [$^3$H]STX was measured as described in Fig. 2 and under Experimental Procedures for treated animals (○) and controls receiving injections of vehicle (●). The results are presented as a Scatchard plot.

day 14 (29). Thus, the loss of norepinephrine after 6-hydroxydopamine treatment occurs more rapidly and recovers more slowly than the loss of STX receptor sites. This time course suggests that 6-hydroxydopamine treatment first causes loss of norepinephrine synthesis and storage, followed more slowly by destruction of a fraction of the nerve membrane. Then, upon recovery, nerve endings reappear followed more slowly by norepinephrine storage granules.

*Effect of reserpine treatment on STX receptor sites in rat heart.* Treatment with 6-hydroxydopamine causes loss of both nerve endings and norepinephrine. In order to determine whether loss of norepinephrine per se is sufficient to cause a reduction of STX receptor site density, rats were treated with reserpine, 4 mg/kg, to deplete norepinephrine stores (30) and then the density of STX receptors in the ventricles was determined. Reserpine (4 mg/kg) was given on days 0, 2, 4, and 6, and animals were killed and assayed on day 7. Depletion of catecholamine stores for 7 days did not have a significant effect on either $K_D$ or $B_{max}$ for STX binding (Fig. 7). Therefore, the effect of 6-hydroxydopamine must be due to loss of autonomic nerve endings rather than depletion of norepinephrine per se.

## DISCUSSION

Our results show that the cardiac ventricles of six vertebrates contain a substantial complement of high-affinity STX/TTX receptor sites whose characteristics are similar to those of nerve and skeletal muscle. These receptor sites are present in all species studied, whether or not cardiac sodium channels studied physiologically have high affinity for TTX. Chicken heart contains 9.3 fmoles of high-affinity STX/TTX receptor sites per milligram, having a $K_D$ of 5 nM for TTX, and sodium channels in heart preparations are blocked with $K_{0.5}$ in the range of 3 nM–20 nM TTX (13–16). In contrast, rat and guinea pig heart have approximately 12 fmoles of high-affinity STX/TTX receptor sites per milligram, with $K_D$ values of 3.5–10.4 nM for TTX, but sodium channels in heart preparations are blocked with $K_{0.5}$ in the range of 1–2 μM TTX (7, 18). These data show that, in general, the high-affinity STX/TTX receptor sites in heart cannot be considered to be associated with the sodium channels responsible for action potential generation in the myocardium.

We have presented evidence that, in bullfrog and rat, at least a major fraction of the high-affinity STX receptor sites in the heart are associated with autonomic nerve endings. In the bullfrog, 28% of the STX receptor sites have $K_D$ values for STX and TTX that are characteristic of frog nerve, and 72% of the STX receptor sites have very low affinity for TTX. We propose that these STX receptors having low affinity for TTX are associated with sodium channels in ventricular myocardium. Physiological studies will be required to determine whether there are indeed functional sodium channels in bullfrog ventricle which have high affinity for STX and low affinity for TTX. Sodium channels in atrial trabeculae of the European grass frog *R. esculenta* are blocked by 100 nM TTX (17) and therefore differ from the STX receptors observed in our binding studies in bullfrog heart. However, some North American frogs and salamanders produce TTX and related toxins (31, 32) and are resistant to their action. The apparent low affinity of sodium channels in bullfrog heart for TTX may represent the remains of an evolutionary relationship with these toxin-producing amphibians.

In the rat, we have found that 50–60% of the high-affinity STX receptor sites in the ventricles are lost on chemical sympathectomy with 6-hydroxydopamine. These data show that at least 50–60% of the high-affinity STX receptors are located on adrenergic nerve terminals. In fact, several considerations suggest that all of the high-affinity STX receptors in rat heart may be located in nerves. (a) Rat brain has an STX receptor density of 250 fmoles/mg (wet weight).[2] Thus, if nerve terminals comprise 5% of the wet weight of the rat ventricle and have the same concentration of sodium channels as brain, a site density of 12.5 fmoles/mg would be expected from nervous elements. This extent of innervation of the ventricle is not unreasonable. (b) Although 6-hydroxydopamine treatment destroys all of the catecholamine storage vesicles in nerve terminals, it may not destroy the entire nerve axon. Since the effects of 6-hydroxydopamine are most readily observed by determination of catecholamine levels or histofluorescence, retention of a fraction of the nerve trunks and fibers with intact membrane receptor sites for STX/TTX but without functional catecholamine biosynthesis and storage is not in conflict with the data in the literature. Thus, it is likely that some nerve membrane remains in the ventricle after 6-hydroxydopamine treatment and contributes to the remaining 40–50% of the high-affinity STX receptor sites. (c) Physiological data indicate that there are no sodium channels with high affinity for TTX in any mammalian heart preparation studied to date. (d) Finally, rat heart cells maintained in cell culture have fewer than 15% as many

[2] W. A. Catterall and J. Coppersmith, unpublished data.

high-affinity STX receptor sites as hearts *in vivo* (18). Since nerve endings will not survive in cell culture without the cell bodies, these data also support the view that the high-affinity STX receptors in heart are located in nerve membranes. Taken together, these several lines of evidence lead us to conclude that all of the high-affinity STX/TTX receptor sites observed in rat heart are likely to be associated with nervous elements and that the myocardial sodium channels have low affinity for these two toxins. The physiological data collected in Table 1 suggests that this conclusion can be extended to all mammals studied to date. Therefore, it appears that STX and TTX will not be useful probes in studies of the localization or molecular properties of sodium channels in mammalian heart.

Physiological studies of chick heart indicate that myocardial sodium channels have high affinity for TTX (12–16). However, chick ventricles have a similar density of high-affinity STX/TTX receptor sites as mammalian ventricles. Since chick ventricles have both cholinergic and adrenergic innervation (27), it seems probable that a substantial fraction of the high-affinity STX receptors in chick ventricle is associated with nerve fibers. It will be difficult to distinguish nerve and myocardial receptors by 6-hydroxydopamine treatment, since chickens are less susceptible to the drug and the cholinergic fibers will be unaffected. Thus, biochemical studies of sodium channels in chick heart using STX and TTX as probes will also be hampered by the presence of nerve sodium channels. Of the species studied, only in bullfrog can nerve and myocardial sodium channels be distinguished in STX/TTX-binding experiments.

While this paper was in preparation, Lombet *et al.* (24) reported binding of a TTX derivative to rat heart membranes. They found that the binding characteristics were very similar to those previously described for nerve and skeletal muscle and concluded that the TTX receptor site on myocardial sodium channels is very similar to that on nerve and skeletal muscle sodium channels (24). In contrast, our results, taken together with the available physiological data, indicate that these high-affinity TTX receptor sites in rat heart resemble those in nerve because they are actually located in nerve endings and that sodium channels in the myocardium have much lower affinity for TTX and STX and therefore are not detected in binding experiments in mammalian heart.

## REFERENCES


1. Reuter, H. Properties of two inward membrane currents in the heart. *Annu. Rev. Physiol.* **41**:413–424 (1979).
2. Hodgkin, A. L., and A. F. Huxley. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* **117**:500–544 (1952).
3. Colatsky, T. J., and R. W. Tsien. Sodium channels in rabbit cardiac Purkinje fibers. *Nature (Lond.)* **278**:265–268 (1979).
4. Lee, K. S., T. A. Weeks, R. L. Kao, N. Akaike, and A. M. Brown. Sodium currents in single heart muscle cells. *Nature (Lond.)* **278**:269–271 (1979).
5. Colatsky, T. J. Voltage clamp measurements of sodium channel properties in rabbit cardiac Purkinje fibers. J. Physiol. *(Lond.)* **305**:215–234 (1980).
6. Narahashi, T. Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54**:813–889 (1975).
7. Baer, M., P. M. Best, and H. Reuter. Voltage dependent action of tetrodotoxin in mammalian cardiac muscle. *Nature (Lond.)* **263**:344–345 (1976).
8. Couraboeuf, E., E. Deroubaix, and A. Coloumbe. Effect of tetrodotoxin on action potentials of the conducting system of dog heart. *Am. J. Physiol.* **236**: H561–H567 (1979).
9. Dudel, J., K. Peper, R. Rudel, and W. Trautwein. The effect of tetrodotoxin on the membrane current in cardiac muscle Purkinje fibers. *Pfleugers Arch. Gesamte Physiol. Menschen Tiere* **295**:213–226 (1967).
10. Scholz, H. Ca-abhangige Membranpotentialanderungen am Herzen und ihre Bedeutung fur die elektro-mechanische Kopplung. Versuche mit Tetrodotoxin in Na-hattigen Losungen. *Naunyn Schmiedeberg's Arch. Pharmakol.* **265**:187–204 (1969).
11. Bean, B. P., C. J. Cohen, T. J. Colatsky, and R. W. Tsien. Concentration dependence of tetrodotoxin action on sodium currents and maximum upstroke velocity in rabbit cardiac Purkinje fibers. *J. Physiol. (Lond.)* **305**:23P–24P (1980).
12. Iijima, T., and A. J. Pappano. Ontogenetic increase of the maximal rate of rise of the chick embryonic heart action potential: relationship to voltage, time, and tetrodotoxin. *Circ. Res.* **44**:358–368 (1979).
13. Galper, J., and W. A. Catterall. Developmental changes in sensitivity of embryonic heart cells to tetrodotoxin and D600. *J. Cell Biol.* **67**:129a (1975).
14. Galper, J., and W. A. Catterall. Developmental changes in the sensitivity of embryonic heart cells to tetrodotoxin and D600. *Dev. Biol.* **65**:216–227 (1978).
15. Fosset, M., J. DeBarry, M. C. Lenoir, and M. Lazdunski. Analysis of molecular aspects of $Na^+$ and $Ca^{2+}$ uptakes by embryonic cardiac cells in culture. *J. Biol. Chem.* **252**:6112–6117 (1977).
16. Couraud, F., H. Rochat, and S. Lissitzky. Stimulation of sodium and calcium uptake by scorpion toxin in chick embryo heart cells. *Biochim. Biophys. Acta* **433**:90–100 (1976).
17. Rougier, O., G. Vassort, and R. Stampfli. Voltage clamp experiments on frog atrial heart muscle fibers with the sucrose gap technique. *Pfleugers Arch. Gesamte Physiol. Menschen Tiere* **301**:91–108 (1968).
18. Catterall, W. A., and J. Coppersmith. Pharmacological properties of sodium channels in cultured rat heart cells. *Mol. Pharmacol.* **20**:533–542 (1981).
19. Ritchie, J. M., and R. B. Rogart. The binding of saxitoxin and tetrodotoxin to excitable tissue. *Rev. Physiol. Biochem. Pharmacol.* **79**:1–51 (1977).
20. Agnew, W. S., A. C. Moore, S. R. Levinson, and M. A. Raftery. Identification of a large molecular weight peptide associated with a tetrodotoxin binding protein from the electroplax of *Electrophorus electricus*. *Biochem. Biophys. Res. Commun.* **92**:860–866 (1980).
21. Barchi, R. L., S. A. Cohen, and L. E. Murphy. Purification from rat sarcolemma of the saxitoxin-binding component of the excitable membrane sodium channel. *Proc. Natl. Acad. Sci. U. S. A.* **77**:1306–1310 (1980).
22. Hartshorne, R. P., and W. A. Catterall. Purification of the saxitoxin receptor of the voltage-sensitive sodium channel from mammalian brain. *Neurosci. Abstr.* **6**:174 (1980).
23. Hartshorne, R. P., and W. A. Catterall. Purification of the saxitoxin receptor of the sodium channel from rat brain. *Proc. Natl. Acad. Sci. U. S. A.* **78**: 4620–4624 (1981).
24. Lombet, A., J. F. Renaud, R. Chicheportiche, and M. Lazdunski. A cardiac tetrodotoxin binding component: biochemical identification, characterization, and properties. Biochemistry **20**:1279–1285 (1981).
25. Ritchie, J. M., R. B. Rogart, and G. R. Strichartz. A new method of labeling saxitoxin and its binding to nonmyelinated fibers of the rabbit vagus, lobster walking leg, and garfish olfactory nerves. *J. Physiol. (Lond.)* **261**:477–494 (1976).
26. Catterall, W. A., C. S. Morrow, and R. P. Hartshorne. Neurotoxin binding to receptor sites associated with voltage-sensitive sodium channels in intact, lysed, and detergent solubilized brain membranes. *J. Biol. Chem.* **254**:11379–11387 (1979).
27. Bolton, T. B. Intramural nerves in ventricular myocardium of the domestic fowl and other animals. *Br. J. Pharmacol. Chemother.* **31**:253–268 (1967).
28. Thoenen, H., and J. P. Tranzer. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn Schmiedeberg's Arch. Pharmakol.* **261**:271–288 (1968).
29. Goldman, H., and D. Jacobowitz. Correlation of norepinephrine content with observations of adrenergic nerves after a single dose of 6-hydroxydopamine in the rat. *J. Pharmacol. Exp. Ther.* **176**:119–133 (1971).
30. Meisheri, K. D., T. E. Tenner, and J. H. McNeill. Reserpine-induced supersensitivity to the cardiac effects of agonists. *Life Sci.* **24**:473–480 (1979).
31. Brown, M. S., and H. S. Mosher. Tarichatoxin: isolation and purification. *Science (Wash. D. C.)* **140**:295–296 (1963).
32. Kim, Y. H., G. B. Brown, H. S. Mosher, and F. A. Fuhrman. Tetrodotoxin: occurrence in Atelopid frogs of Costa Rica. *Science (Wash. D. C.)* **184**:151–152 (1975).


**Send reprint requests to:** Dr. William A. Catterall, Department of Pharmacology, University of Washington, Seattle, Wash. 98195.