EFFECTS OF YOHIMBINE ON SQUID AXONS

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ABSTRACT Yohimbine, an indolealkylamine alkaloid, reduces the amplitude of the sodium current in the squid giant axon. For doses that reduce sodium current amplitude by up to 50%, there is no significant change in the kinetics or in any of the voltage-dependent parameters associated with sodium channels. The effective equilibrium constant for yohimbine binding to the sodium channel is $\sim 3 \times 10^{-4}$ M.

Repetitive depolarizing pulses increase the inhibition of squid axon sodium current by yohimbine. This use-dependent inhibition is enhanced by increasing the concentration of yohimbine, by increasing the frequency of pulsing, and by increasing the magnitude or the duration of depolarization. It is reduced by hyperpolarizing prepulses. This behavior can be explained by a model wherein yohimbine binds more readily to open sodium channels than to closed sodium channels and wherein the Hodgkin-Huxley kinetic parameters are modified by the binding of the drug. This type of model may also explain the tonic and use-dependent inhibition previously described by others for local anesthetics.

INTRODUCTION

Yohimbine is a naturally occurring indolealkylamine alkaloid which, like reserpine (another indolealkylamine alkaloid), can be isolated from many Rauwolfia species of the family Apocynaseae. Although reserpine is the most studied and the most clinically used, yohimbine has an extensive literature, and the generally recognized effects of yohimbine are alpha-adrenergic blockade, blockade of peripheral 5-hydroxytryptamine receptors, various central nervous system effects (including antidiuresis, vasopressin release, and a general picture of anxiousness), and various peripheral autonomic nervous system effects (Goodman and Gilman, 1975). Yohimbine is currently available in several prescription preparations and has suggestive evidence of efficacy in the treatment of male impotence (Roberts and Sloboda, 1974). The structural formulas for yohimbine and reserpine are shown in Fig. 1.

Although yohimbine is best known for its alpha-adrenergic blockade (Hamet, 1925; Nickerson, 1949; Werner, 1958), it also has central effects that alter cardiovascular response (Lang et al., 1975) and has effects on the intermediary metabolism of tryptophan (Sourkes et al., 1969; Papeschi et al., 1971). Thus its effects are rather ubiquitous and involve many organ systems as well as subcellular responses.

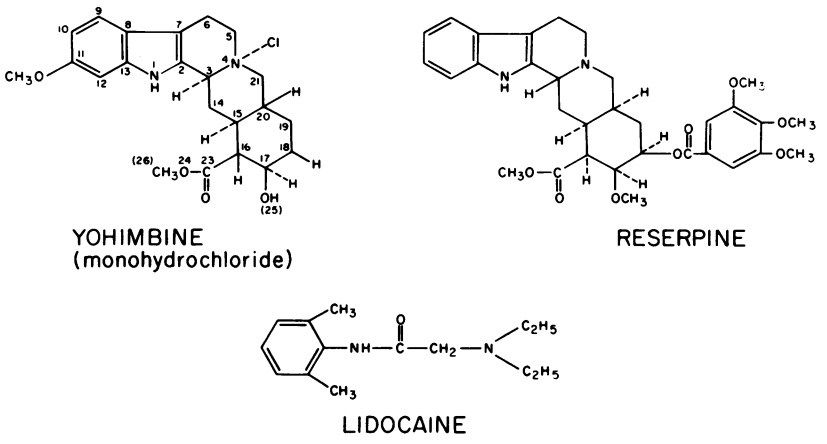


FIGURE 1 Structural formulas of yohimbine, reserpine, and lidocaine.

Of particular interest to us is yohimbine's effects on electrically excitable membranes. As early as 1908 (Tait and Gunn, 1908), demonstrated effects of yohimbine on electrically excitable membranes were noted to depend upon the past history of the preparation. Subsequently, a number of studies in isolated frog skin (Holman and Shaw, 1955); toad (*Bufo marinus*) nerve and muscle (Shaw et al., 1955; Simon, 1955); and frog sciatic nerve (Shanes, 1951; Graham, 1935; Doty and Gerard, 1950) collectively demonstrated that concentrations of yohimbine from 2.6×10^{-5} to 2.6×10^{-3} M had little or no effect on resting membrane potentials, ion concentration gradients, or oxygen consumption, but did markedly affect the ability of the tissues to sustain repetitive action potentials and decreased the oxygen consumption of stimulated nerves. These observations suggest a "use-dependent" effect of yohimbine on electrically excitable membranes.

Local anesthetics such as procaine and procaine amide (Courtney, 1975), as well as tertiary and quarternary derivatives of lidocaine (Strichartz, 1973; Courtney, 1975; Khodorov et al., 1976; Hille, 1977) have been demonstrated to have use-dependent effects on sodium channels in frog myelinated nerve. The structural formula for lidocaine is shown in Fig. 1. The effects of yohimbine, a compound structurally dissimilar from the local anesthetics but with demonstrated use-dependent effects, seemed a promising avenue of investigation.

In this paper we will describe the inhibition of squid axon sodium current by yohimbine. Other experiments on the inhibition of sodium current by yohimbine in the presence of batrachotoxin were performed on tissue-cultured neuroblastoma cells; these have recently been published (Huang et al., 1978).

In the Discussion we will describe a model for the action of yohimbine based on both the inhibition data reported in this paper and the data on neuroblastoma cells referred to above. Also, we will compare the experimental data for yohimbine with experimental data obtained for lidocaine derivatives and other local anesthetics. A preliminary report of this work was recently presented (Lipicky et al., 1977).

METHODS

The isolated caudalmost, stellar nerve of *Loligo pealei* was isolated by conventional methods (Arnold et al., 1974) from squid at the Marine Biological Laboratory, Woods Hole, Mass. Cleaned axons were mounted horizontally in a Lucite chamber similar to that described by Fishman (1970). Temperature of the superfusing sea water was monitored in the inlet passage near the axon and was taken as the axon temperature. Chamber volume amounted to ~1.5 ml and was continuously exchanged at a superfusion rate of ~3.5 ml/min.

The axons were internally perfused through a glass cannula (Tasaki et al., 1962). Another cannula was used for measuring the internal potential (Fishman, 1973). Attached to this internal electrode was a platinized platinum wire that acted as current source in the voltage clamp feedback loop (Chandler and Meves, 1965; Rojas and Ehrenstein, 1965). Insertion and subsequent withdrawal of the perfusion cannula was aided by pronase treatment (Bezanilla and Armstrong, 1972). The concentration of pronase (Caliochem, Los Angeles, Calif.) used during withdrawal of the perfusion cannula was 0.3 mg/ml. Withdrawal was as rapid as practical and an internal perfusion solution without pronase was begun within seconds of reaching the final positioning of the internal electrodes. The voltage-clamp system was similar to that described by Fishman (1970).

All measurements were made using standard step-clamp procedure (Moore and Cole, 1963). Absolute membrane potentials have been used throughout the paper. Holding potentials were between −58 and −62 mV. Currents were photographed on 35-mm film (Grass camera, Grass Instrument Co., Quincy, Mass.) from a Tektronix 7313 storage oscilloscope (Tektronix, Inc., Beaverton, Ore.). Leakage current was measured for hyperpolarizing pulses, and assumed to be linear with respect to membrane potential. All currents are reported with this leakage current subtracted. All error bars represent one standard error. When multiple pulses were delivered to the axon, the pulses were counted on a Baird Atomic Model 197 nuclear scaler (Baird Atomic, Inc., Bedford, Mass.) and manually recorded to coincide with the current traces that were stored on the Tektronix oscilloscope.

The external solution in all cases had the following ionic composition: 430 mM sodium chloride, 50 mM magnesium chloride, 10 mM calcium chloride, and 5 mM Tris, titrated to pH 7.4 at 25°C. This gives a pH of ~8.0 at the temperatures used for experiments. Temperatures for any axon were held constant (i.e., ±0.1°C) and ranged from 3° to 8°C from experiment to experiment. The internal perfusion solution had the following ionic composition: 0.5 M potassium chloride, buffered with 5 mM Tris (titrated to pH 7.4 at 25°C).

Weighed amounts of yohimbine monohydrochloride (mol wt 390.5) were dissolved directly in either the external or internal solutions for the highest concentrations (2.6×10^{-4} M), near the limit of solubility of yohimbine in sea water. Lower concentrations were made from a stock solution of 2.6×10^{-4} M and appropriately diluted. All solutions were made fresh daily including the yohimbine solution. Yohimbine was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis.

RESULTS

Yohimbine has two phenomenologically different effects. One effect is a reduction in sodium currents observed with depolarizing pulses whose frequency is no higher than 0.1 Hz. This tonic effect is illustrated by Fig. 2, which shows voltage clamp records taken at widely spaced time intervals after addition of yohimbine. Fig. 2 shows that the amplitude of the sodium current is gradually reduced. The second effect—the use-dependent reduction in sodium currents—occurs for higher-frequency depolarizing pulses, and will be considered later in this paper.

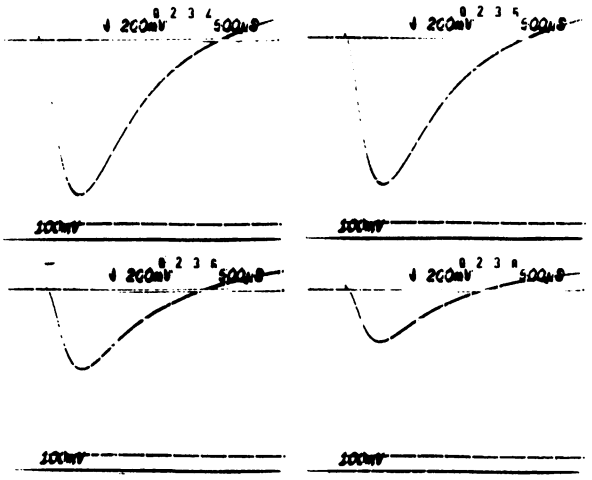


FIGURE 2 Sodium currents with infrequent depolarizing pulses in presence of external yohimbine. Potential during pulse: 0 mV. Pulse duration: 5 ms. Time of pulses: 0, 3, 39, and 48 min. Yohimbine concentration: 2.6×10^{-4} M.

A convenient way to describe the time-course of the reduction in current through sodium channels is to plot the change in peak sodium current. To obtain the peak sodium current, leakage was first subtracted from the experimentally observed maximum current for each pulse. It would also be desirable to subtract the current through potassium channels. To this end, we have performed experiments with tetraethylam-

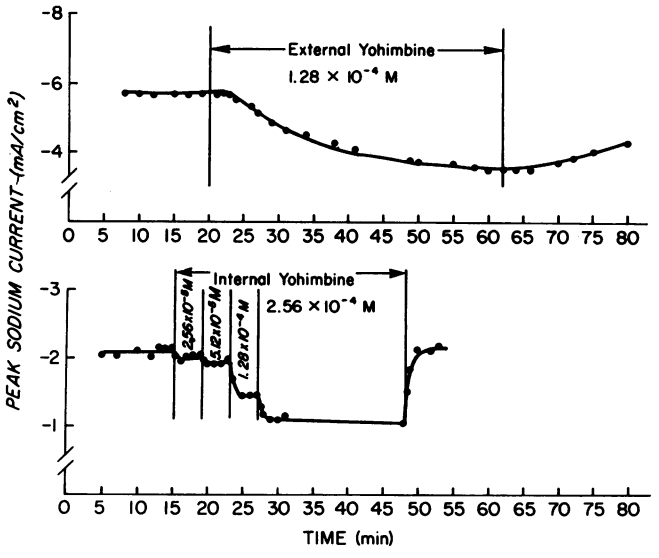


FIGURE 3 Time-course of decline of peak sodium current as observed with infrequent depolarizing pulses during application of the indicated concentrations of yohimbine. Upper and lower curves are for different axons.

monium (TEA), tetramethylammonium (TMA), or cesium added to the internal perfusate to block potassium current. However, each of these agents also affected the sodium currents. For example, TEA significantly reduced the amplitude of the sodium current, and cesium changed its time-course. We, therefore, considered ignoring the potassium current, provided it would not seriously affect our results. Experiments with tetrodotoxin, which selectively blocks sodium channels, showed that at the time of the peak sodium current, in both control and yohimbine-treated axons, the potassium current was <5% of the sodium current for the depolarizing pulses used. Thus, the peak currents without correction for potassium current are a good approximation to the peak sodium currents.

The decrease in the amplitude of peak sodium current for both external and internal application of yohimbine is shown in Fig. 3. For these experiments, the pulse potential was first adjusted to give maximum peak sodium current and was then kept constant. The upper graph of Fig. 3 shows results from an axon with 1.3×10^{-4} M yohimbine applied externally. After a short lag (chamber mixing time ~3 min), the sodium current gradually decreased, with a time constant of ~15 min. The effect was reversible, albeit slowly, and the experiment was discontinued before full reversal. The lower graph of Fig. 3 shows the results of yohimbine applied by internal perfusion. The decrease of sodium current started sooner and the sodium current reached steady state much sooner than for external application of yohimbine. The time constant was ~0.5 min. The effects of internal yohimbine were completely reversible.

A number of experiments were performed to determine whether yohimbine had any significant effect on sodium current, other than decreasing the amplitude. No such effect was found. In particular, the time-to-peak and the shape of the peak *I-V* curve were essentially unchanged by yohimbine at concentrations that reduced the amplitude of sodium current by 40–50%. Also, this concentration of yohimbine did not change the voltage dependence of sodium inactivation, as measured by standard voltage clamp test pulses after 40-ms prepulses of varying amplitude. Neither did this concentration of yohimbine change the time constant of the Hodgkin-Huxley inactivation parameter h at the resting potential, as measured by the dependence of the current amplitude ratio of two identical depolarizing pulses on the interval between them. Thus, the dosages of yohimbine we used seem to change only the amplitude of the sodium current for the tonic effect.

The dose-response relation for the tonic reduction of maximum peak sodium current amplitude is shown in Fig. 4 for internal application of yohimbine. Because of the limited solubility of yohimbine in sea water, the dose-response relation was not extended to higher doses.

For external application of yohimbine, the results were qualitatively similar, but external yohimbine was somewhat more effective than internal yohimbine at lower concentrations. This difference may be accounted for by differences in internal pH between the internal perfusion solution and axoplasm (Boron and DeWeer, 1976). Since the internal perfusion data is based on more controlled conditions, it is used for fitting to theoretical dose-response curves in Fig. 4.

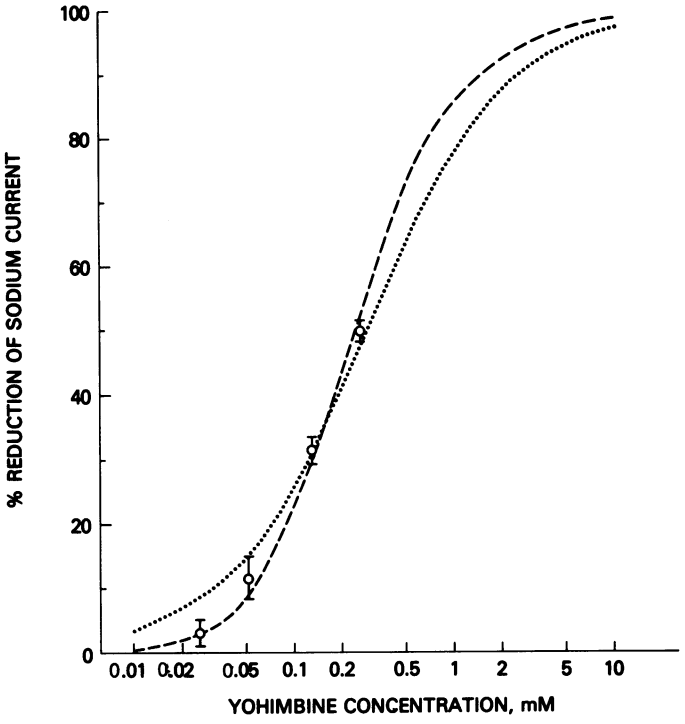


FIGURE 4 Dose-response curve for the tonic reduction of sodium current by internal yohimbine. (. . .) Theoretical curve for one yohimbine molecule per channel. (- - -) Theoretical curve for two yohimbine molecules per channel.

In Fig. 4, the experimental dose-response curve has been fit with theoretical curves corresponding to two assumptions about yohimbine binding. The dotted curve is for the assumption that one yohimbine molecule bound to a channel eliminates the current through one channel, and the dashed curve is for the assumption that two yohimbine molecules must bind to a channel, with equal equilibrium constants, before the channel current is eliminated. The two-site model clearly fits better, but the one-site model cannot be ruled out. From the data available, it is also possible that the sodium current is not completely eliminated by yohimbine. In any event, if the yohimbine effect is caused by binding to either a one-site-per-channel or a two-site-per-channel receptor, then the effective dissociation constant for the overall binding is $\sim 3 \times 10^{-4}$ M.

If the frequency of depolarizing test pulses was below 0.1 Hz, only the tonic effects occurred. For higher frequency depolarizing test pulses, there was an additional decrease in sodium currents that depended on the amplitude, the duration, and the frequency of the test pulses. This use-dependent yohimbine effect is illustrated in Fig. 5, which shows that several action potentials or depolarizing pulses transiently decrease the amplitude of the sodium current beyond the tonic level. Use dependence can also be seen in Fig. 6, which shows the decrease in current amplitude during a series of

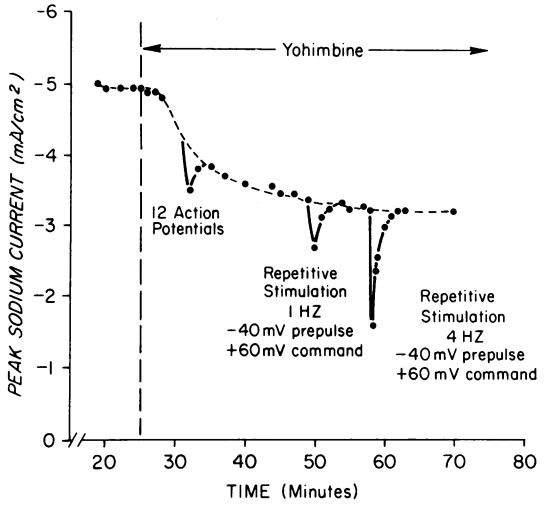
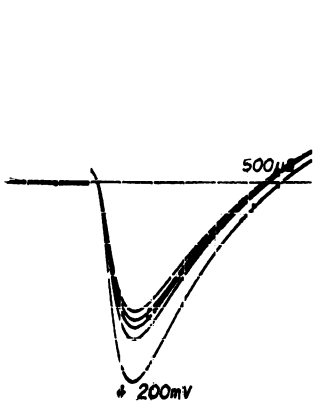


FIGURE 5 Time-course of decline of peak sodium current as observed with infrequent depolarizing pulses (dashed line) and of the further decline caused by repetitive stimulation as indicated. 1.3×10^{-4} M external yohimbine.



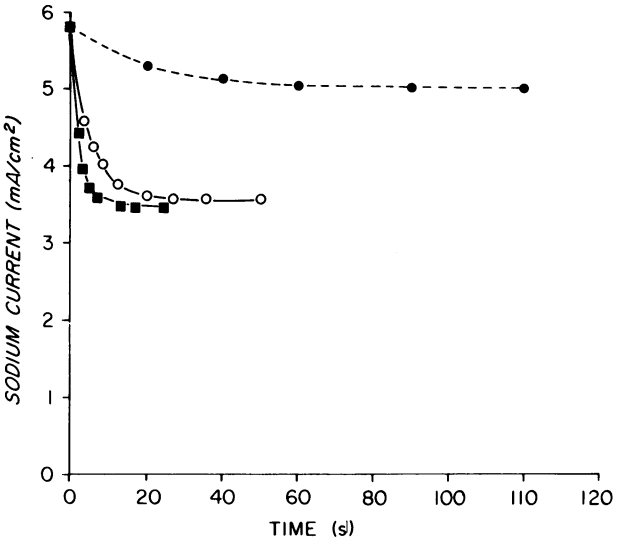


FIGURE 6

FIGURE 7

FIGURE 6 Sodium currents during repetitive pulses in presence of external yohimbine. Yohimbine concentration: 1.3×10^{-4} M. Pulse frequency: 1 Hz. Potential during pulse: 0 mV. Pulse duration: 5 ms. Potential during prepulse: −100 mV. Prepulse duration: 20 ms. Pulses 1, 4, 6, 8, and 12 are shown.

FIGURE 7 Time-course of peak sodium amplitude during repetitive pulsing at several frequencies. Yohimbine concentration: 1.3×10^{-4} M external. Potential during pulse: 0 mV. Pulse duration: 5 ms. Potential during prepulse: −100 mV. Prepulse duration: 20 ms. (●) Frequency: 0.1 Hz. (○) Frequency: 1 Hz. (■) Frequency: 4 Hz.

depolarizing pulses at 1 Hz. In Fig. 6, for clarity, only pulses 1, 4, 6, 8, and 12 are shown.

As expected for reduced sodium currents, action potentials were reduced in magnitude and had a longer latency for each successive depolarization. At sufficiently large drug concentrations ($\sim 3 \times 10^{-5}$ M) and sufficiently high stimulation frequencies (~ 1 Hz), the action potentials were completely abolished after several stimuli.

Fig. 7 shows the time-course of the reduction of the amplitude of sodium currents during repetitive depolarizing pulses for several frequencies with external yohimbine. As shown in Fig. 7, increasing the frequency caused a faster decrease of sodium current. Also, 1 Hz is a frequency high enough to provide maximum decrease of sodium current. Therefore, in the following experiments to characterize the use-dependent effect (Figs. 8–14), the frequency was standardized at 1 Hz.

Experiments on the use-dependent reduction of sodium currents were performed with different concentrations of yohimbine in the internal solution and with a depolarizing pulse amplitude of 60 mV and duration of 2 ms. The resultant dose-response curve is shown in Fig. 8 along with the dose-response curve for the tonic effect. In general, the change in the dose-response curve from the tonic to the use-dependent case depends on the rates for binding and unbinding of the drug to the channel and on the change of opening and closing kinetics caused by drug binding. Since these are not known with much precision, it is not possible to determine quantitatively the theoretical change in the dose-response curve. However, the data in Fig. 8

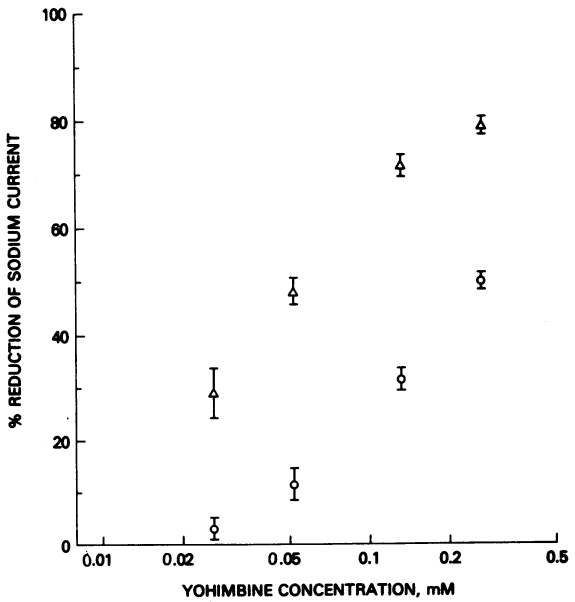


FIGURE 8 Dose-response curves for internal yohimbine. (o) Tonic effect. (Δ) Use-dependent effect. For use-dependent effect, pulse potential is 0 mV, pulse duration is 2 ms, and pulse frequency is 1 Hz.

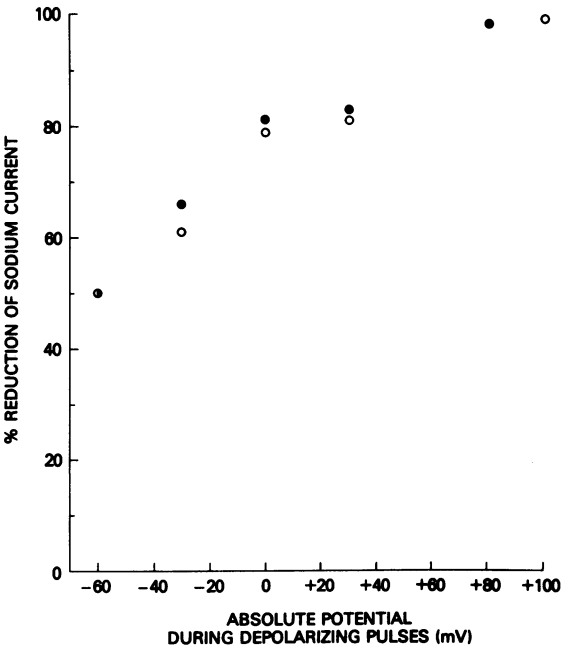


FIGURE 9 The effect of depolarizing pulse potential on the steady-state reduction of sodium current in the presence of yohimbine. Frequency: 1 Hz. Pulse duration: 2 ms. (●) 2.6×10^{-4} M yohimbine in external solution. (○) 2.6×10^{-4} M yohimbine in internal solution.

is qualitatively consistent with the hypothesis that depolarizing pulses effectively cause an increase in the binding of yohimbine to sodium channels.

Fig. 9 shows that increasing the magnitude of depolarizing pulses increases the magnitude of the use-dependent reduction of sodium current for both internal and external application of 2.6×10^{-4} M yohimbine. As was found for the tonic effect at this concentration of yohimbine, there is no significant difference between internal and external yohimbine in the reduction of sodium current. An additional effect of increasing the magnitude of depolarizing pulses is more rapid development of the use-dependent effect, as shown in Figs. 10 and 11.

On the basis of the preceding results, inhibition of sodium current can be enhanced by increasing the concentration of yohimbine, by increasing the frequency of pulsing, and by increasing the amplitude of the depolarizing pulses. An important question is whether this enhancement is caused by the depolarization itself or by the opening of sodium channels brought about by the depolarization. To address this question, we varied the duration of depolarizing pulses of fixed amplitude and frequency, and measured the steady-state reduction in the amplitude of sodium current. The results are shown in Fig. 12, which shows that increasing the duration up to ~1 or 2 ms causes a large increase in use-dependent inhibition, but that further increases of pulse duration do not have much effect. Similar results were obtained when axons were perfused with CsF to eliminate potassium current.

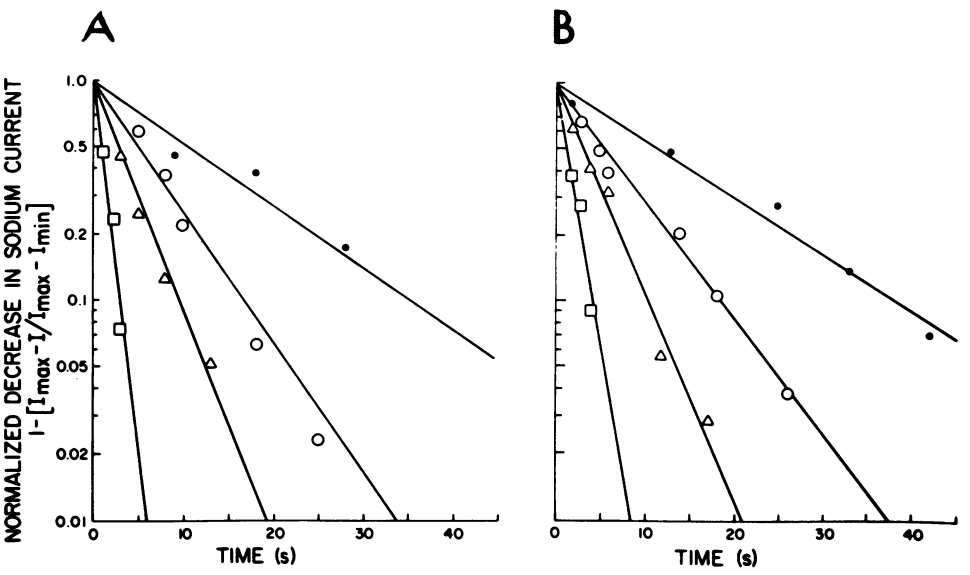


FIGURE 10 Time course of sodium current reduction during 1-Hz repetitive pulsing. 5-ms pulses. Semi-logarithmic scale. (*A*) In presence of 2.6×10^{-4} M internal yohimbine. (*B*) In presence of 2.6×10^{-4} M external yohimbine. (●) −30 mV potential during depolarizing pulses. (○) 0 mV potential during depolarizing pulses. (△) +30 mV potential during depolarizing pulses. (□) +100 mV potential during depolarizing pulses.

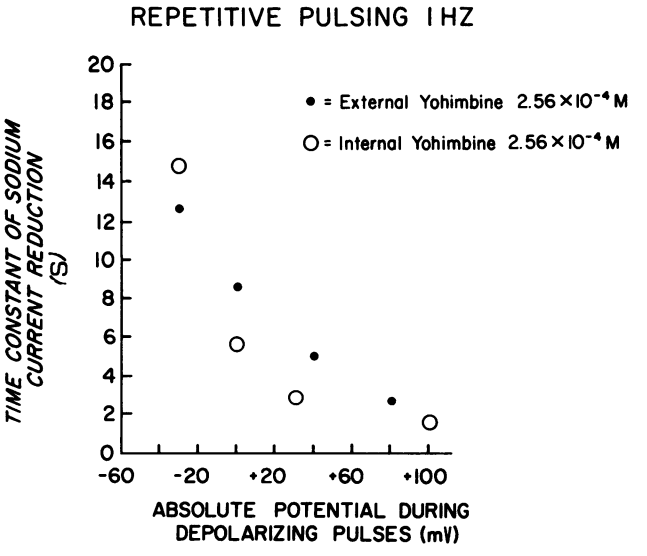


FIGURE 11 Time constant for use-dependent reduction of sodium current as a function of potential during depolarizing pulse. Frequency: 1 Hz. Pulse duration: 2 ms. (○) 2.6×10^{-4} M internal yohimbine. (●) 2.6×10^{-4} external yohimbine.

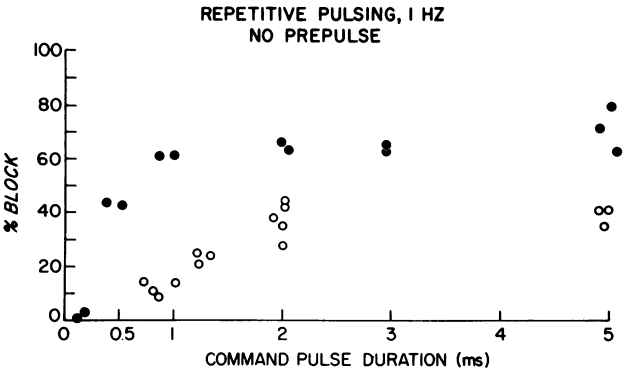


FIGURE 12 Effect of depolarizing pulse duration on the steady-state use-dependent reduction of sodium current in the presence of 2.6×10^{-4} M internal yohimbine. Frequency of pulsing: 1 Hz. Pooled data from four axons. (○) −20 mV potential during depolarizing pulse. (●) +20 mV potential during depolarizing pulse.

To find out whether inactivation is altered by the use-dependent reduction of sodium current, we used pulse sequences similar to those used in the measurement of inactivation for the tonic reduction of sodium current, but applied them continuously at a frequency of 1 Hz. In Fig. 13 the results are plotted in the form of standard inactivation curves. The solid curve is the tonic inactivation curve, determined from the first pulses of each sequence. The dashed curve shows the steady state achieved at 1 Hz. According to standard practice, both curves are normalized.

Although the triangles in Fig. 13 appear to be part of a standard inactivation curve,

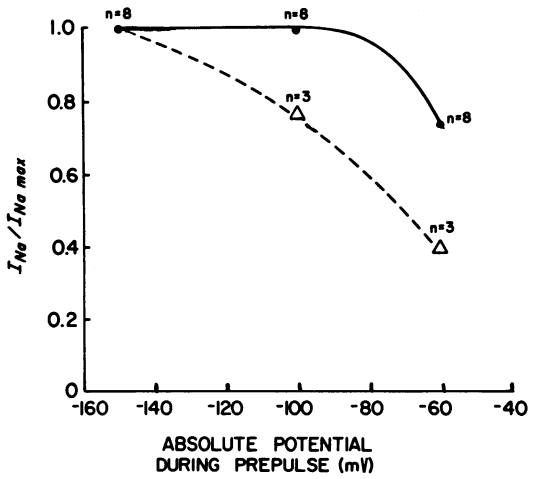


FIGURE 13 Inactivation curves before and after pulsing at 1 Hz. 1.3×10^{-4} M external yohimbine. Prepulse duration: 40 ms. Potential during test pulse: 0 mV. Curves through experimental points are drawn by eye. (●) Ratios of initial test pulses. (△) Ratios after pulsing at 1 Hz.

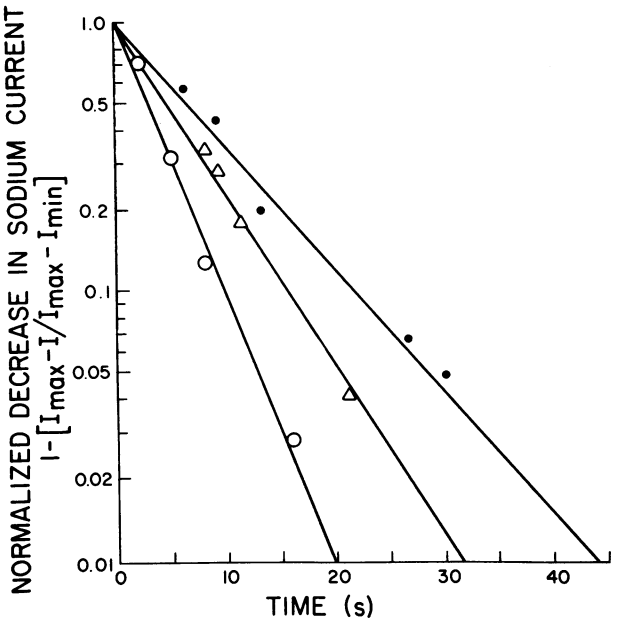


FIGURE 14 Time-course of sodium current reduction during pulsing at 1 Hz. Same conditions as Fig. 13. (●) No prepulse. (△) −100 mV potential during prepulse. (○) −150 mV potential during prepulse.

there is an ambiguity in interpreting these points, because the current corresponding to each triangle in Fig. 13 is reduced by two causes—by inactivation and by use-dependent inhibition. Since the prepulses during repetitive pulsing were different for each point, the amount of use dependence may also be different. Thus, it is not possible from these experiments to determine how much of the change shown in Fig. 13 is caused by changes in inactivation and how much is caused by changes in use-dependent inhibition.

As previously indicated, the voltage dependence of inactivation is not changed even for a 50% tonic decrease of sodium current by yohimbine. Thus, a considerable simplification is entailed by interpreting Fig. 13 as a change in use-dependent inhibition, since this interpretation does not require separate explanations for the tonic and use-dependent effects.

Regardless of the interpretation of Fig. 13, the time to reach steady state can be plotted unambiguously, and was found to decrease as prepulse potential was increased (cf. Fig. 14 and Table I).

DISCUSSION

For the tonic reduction of sodium current caused by yohimbine, we have not found any significant change in kinetics as reflected in the time-to-peak, the voltage dependence of sodium inactivation, or the time constant of inactivation. This is the same

TABLE I

USE-DEPENDENT INHIBITION OF SODIUM CURRENT BY SEVERAL DRUGS

	Quaternary lidocaine derivative QX-314	Tertiary lidocaine derivative GEA-968	Yohimbine
Preparation	Frog node	Frog node	Squid axon
Reference	Strichartz (1973)	Courtney (1975)	This paper
Effect of increasing amplitude of depolarizing pulses on inhibition	More inhibition	More inhibition	More inhibition
Is continuous depolarization as effective as short pulses for the same total time?	No	No	No
Effect of increasing amplitude of hyperpolarizing prepulses on inhibition		More or less inhibition, depending on voltage range	Less inhibition
Effect of increasing amplitude of hyperpolarizing prepulses on time to reach steady state	Faster		Faster
Effect of increasing amplitude of hyperpolarizing pulses on time to remove use-dependent inhibition	Faster		

type of behavior found for tetrodotoxin and saxitoxin, drugs now known to occlude sodium channels (Narahashi et al., 1967). Thus a possible mechanism to explain the effects of yohimbine is that it also occludes sodium channels. For tetrodotoxin and saxitoxin, however, the evidence for occlusion is based on a variety of additional experimental data. Bezanilla and Armstrong (1974) showed that tetrodotoxin does not affect gating current; Henderson et al. (1974) showed that tetrodotoxin and saxitoxin bind at the same site as do cations; and Catterall (1975) showed that tetrodotoxin is a noncompetitive inhibitor of the action of veratridine and batrachotoxin. In the absence of experimental data of this type on yohimbine, occlusion can only be regarded as a possible mechanism.

Recent work on use dependence of local anesthetics has stressed a model wherein the local anesthetics not only occlude sodium channels, but also change voltage-dependent gating parameters of the channels (Courtney, 1975; Strichartz, 1976; Hille, 1977). This is another possibility for explaining inhibition of sodium current by yohimbine.

Still another possibility is that yohimbine might bind to sodium channels without occluding them, and modify channel kinetics in such a way that the peak sodium current for the drug-bound channels is much smaller than the peak sodium current for unbound channels. This would certainly reduce peak sodium current. It might be expected that this mechanism would also cause a significant change in the observed sodium kinetics. However, this may not be the case, even for observed changes in sodium current amplitude of 50%. The reason is that the modified channels may contribute much less to the observed overall current than the unmodified channels, even if they are approximately equal in number. For example, if a given number of modified

channels provide 10% of the peak current provided by the same number of normal channels, and if there is a 50–50 mixture of modified and unmodified channels, then the observed overall current will be ~55% of normal. The unmodified channels would contribute 50% of normal current and the modified channels would contribute 5% of normal current. With this weighting, the modification of channel kinetics may not be observable. Thus, the tonic results could be explained by occlusion, by modified channel gating, or by a combination of both effects.

In a recent paper, Huang et al. (1978) demonstrated that in tissue-cultured neuroblastoma cells yohimbine is a competitive inhibitor of batrachotoxin—a drug known to keep sodium channels open by affecting the gating mechanism (Albuquerque et al., 1971). This is strong evidence that yohimbine affects the gating mechanism, as required by the modified-channel-kinetics model. By contrast, the occlusion model predicts noncompetitive inhibition between yohimbine and batrachotoxin, and the mixed model predicts a large component of noncompetitive inhibition. One of the purposes of this paper is to test whether the use-dependent effects of yohimbine on squid axon sodium channels are consistent with the modified-channel-kinetics model required to explain the neuroblastoma results. This is important, since previous work on use dependence has been focused on testing consistency with the occlusion model.

For some derivatives of lidocaine, Strichartz (1973) has presented evidence that the observed use dependence is based on the opening of sodium channels by repetitive depolarization. We have tested whether the observed use dependence in yohimbine also occurs because of the opening of sodium channels. Fig. 12 shows the dependence of inhibition on pulse duration and on magnitude of depolarization. The inhibition increases as the pulse duration increases up to ~1–2 ms, about the time the channel stays open during voltage clamp, but saturates for longer depolarizing pulses. Also, for smaller depolarization, where the sodium channels open more slowly, a longer duration is required before saturation occurs. These observations are consistent with the notion that open channels are responsible for the enhanced inhibition.

There are basically two ways that the reduction of sodium current by a drug can depend on whether the channels are open. One way is for the drug to occlude sodium channels. In this case, only open channels can admit the drug into the lumen so that occlusion can take place. The other way is for the drug to bind outside the lumen and modify channel kinetics in such a way as to decrease peak sodium current. This process could also be enhanced by the presence of open channels. Such an enhancement would not be caused by the openness of the channel, per se, but because the overall conformation of an open channel happens to promote drug binding. A similar enhancement has been discussed by Hille (1977) in relation to binding within the lumen.

A useful way to compare these two mechanisms is to consider the overall scheme for drug binding and unbinding and channel opening and closing:

$$\begin{array}{ccc} C_c & \underset{H\text{-}H}{\rightleftharpoons} & C_o \\ & & \uparrow\downarrow \\ C_c \cdot Y & \rightleftharpoons & C_o \cdot Y \end{array}$$

where C_c and C_o represent closed and open channels which obey Hodgkin-Huxley (H-H) kinetics, Y represents yohimbine, and $C_c \cdot Y$ and $C_o \cdot Y$ represent closed and open yohimbine-bound channels that obey some modified form of Hodgkin-Huxley kinetics. This general scheme was explicitly used by Strichartz (1973) to describe the occlusion model. He correctly pointed out that for an occlusion model only the C_o channels are conducting. For the modified-channel-kinetics model, both C_o and $C_o \cdot Y$ channels are conducting. From this point of view, the main difference between the occlusion and modified-channel-kinetics mechanisms is whether bound, open channels are conducting or not.

For occlusion, the binding process itself blocks channels and must cause a conductance decrease. For the modified-channel-kinetics model, the diagram shown in the previous paragraph can be regarded as a description of two parallel processes. Unbound channels follow H-H kinetics, and drug-bound channels follow a modified version of H-H kinetics. Depending on the specific changes in kinetic parameters caused by the binding of the drug, the maximum fraction of drug-bound channels that open during a depolarizing pulse could be larger or smaller than the maximum fraction of unbound channels that open during the same pulse. Thus, modified channel kinetics could lead to inhibition or enhancement of peak sodium current. The experimental observation that yohimbine causes inhibition of sodium current constraints the kinetic parameters for drug-bound channels in the modified-channel-kinetics model.

During the development of use-dependent inhibition, there is a small increase in the the time-to-peak of the sodium current. In Fig. 6, it is ~ 100 μs. This is not primarily a series resistance artifact, since our calculation of sodium current vs. time curves using the Hodgkin-Huxley equations indicates that the shift in time-to-peak caused by a series resistance of 5 $\Omega \cdot cm^2$ is ~ 10 μs. Therefore, in simulating use-dependent inhibition, we sought parameters that would provide a significant decrease in the amplitude of the sodium current, and a small shift to the right of the time-to-peak.

Fig. 15 is a computer simulation of a particular version of modified-channel-kinetics, where the only Hodgkin-Huxley parameter modified by yohimbine is α_m. In this case, α_m is decreased fivefold. The values of other parameters used in the calculations are listed in the legend to Fig. 15. The calculated curves of Fig. 15 are quite similar to the experimental results of Fig. 6, demonstrating that the modified-channel-kinetics model can explain the effect of repetitive pulsing with yohimbine. The example of Fig. 15 is not meant to suggest that the chosen parameters are the correct ones. Other combinations of parameters may provide a better fit and it may be possible to determine additional experimental constraints to distinguish between possible sets of modified-channel-kinetics parameters. The example of Fig. 15 is simply meant to indicate qualitatively that some form of modified channel kinetics can explain the large decrease in amplitude and the small shift in time-to-peak.

Table I qualitatively summarizes representative results of this paper concerning the effects of use dependence, and compares them with corresponding results for two derivatives of lidocaine. A comparison between yohimbine and QX-314 shows considerable similarity. One possible difference relates to the effect of hyperpolarizing

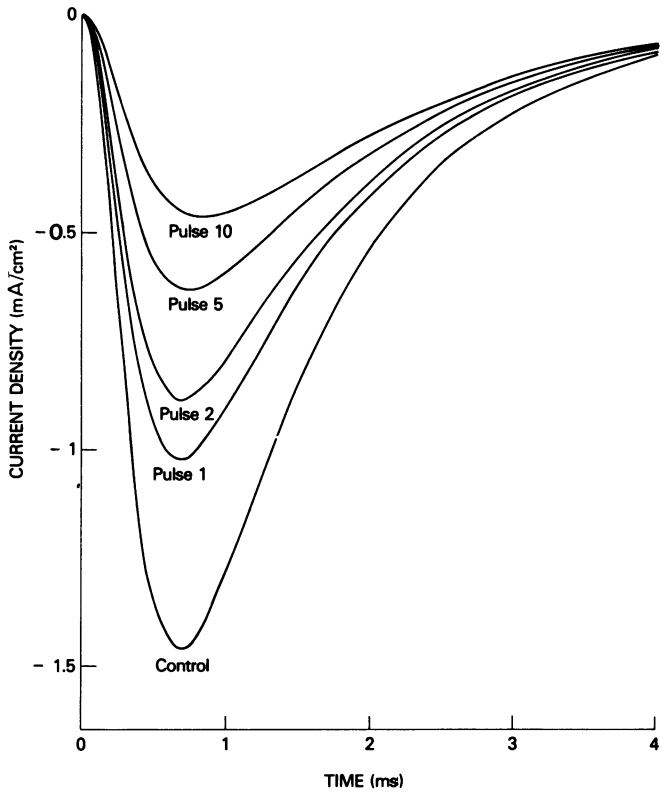


FIGURE 15 Computer simulation of time-course of sodium current during repetitive depolarizing pulses with yohimbine for model described in text with following parameters: Potential during depolarizing pulse ... 0 mV. Pulse duration ... 4 ms. Pulsing frequency ... 4 Hz. Kinetic parameters for unbound channels and for drug-bound channels at rest are standard H-H parameters. Kinetic parameters for drug-bound channels at 60 mV depolarization ... α_m is decreased fivefold, and all other parameters are standard H-H parameters. Rate coefficient for drug binding to open channel = 0.5 ms^{-1}. Rate coefficient for drug unbinding from open channel at −60 mV = 1.17 ms^{-1}. Rate coefficient for drug unbinding from open channel at 0 mV = 0.19 ms^{-1}.

prepulses on use-dependent inhibition. As previously indicated, the yohimbine results of Fig. 13 can be interpreted as a change in use-dependent inhibition. According to this interpretation, hyperpolarizing prepulses of increasing amplitude cause less use-dependent inhibition. For QX-314, by contrast, hyperpolarizing prepulses of increasing amplitude seem to cause more use-dependent inhibition. However, since steady-state block is not reached in the QX-314 experiments, this point is somewhat ambiguous, and the corresponding box in Table I is left blank. Regarding this property, GEA-968 occupies a middle ground, causing more or less inhibition in different voltage ranges.

Another comparison that can be made among the several drugs involves a tonic property, and is not included in Table I: Does tonic inhibition alter the voltage de-

pendence of inactivation? As indicated previously, it does not for yohimbine. Courtney (1975) also demonstrated that it does not for GEA-968. On the other hand, Hille (1977) demonstrated a marked voltage shift in the hyperpolarizing direction for both lidocaine and benzocaine.

Overall, the effects of yohimbine on sodium currents are rather similar to those of the local anesthetics. There may be some differences, but these are comparable to the differences among the several local anesthetics.

We have shown that our results with yohimbine on squid axons are consistent with the modified-channel-kinetics model, required to explain the competitive inhibition results between yohimbine and batrachotoxin found in neuroblastoma cells by Huang et al. (1978). It may also be possible to determine the mechanism for inhibition of sodium current by some local anesthetics by testing whether these local anesthetics and batrachotoxin are competitive inhibitors. Preliminary evidence (Khodorov et al., 1976) indicates that procaine and batrachotoxin are competitive inhibitors. This suggests that the modified-channel-kinetics model may also apply to procaine.

An interesting aspect of the modified-channel-kinetics model is that the binding of the drug does not occur at the polar interior of the sodium channel, as required by occlusion models. The actual site of binding is not well specified by the modified-channel-kinetics model, but it could be at a hydrophobic portion of the channel protein. This would be consistent with the considerable body of experimental data correlating anesthetic potency with lipid solubility.

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