INTERACTION BETWEEN BATRACHOTOXIN AND YOHIMBINE

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ABSTRACT The neurotoxins, batrachotoxin and veratridine, are specific activators of sodium channels and cause an increase in the rate of ^{22}Na uptake in neuroblastoma cells. Yohimbine, an indolalkylamine alkaloid, inhibits this batrachotoxin-induced ^{22}Na uptake. The dose-response curve of yohimbine suggests that the inhibitor acts reversibly on a single class of binding sites with dissociation constant of $3–4 \times 10^{-5}$ M. The dissociation constant is not affected by depolarization from −41 to 0 mV. Kinetic and equilibrium experiments indicate that yohimbine is a competitive inhibitor of the action of batrachotoxin. These results support the conclusion that yohimbine inhibits the sodium flux by acting on the channel gating mechanism rather than by occluding the channels.

INTRODUCTION

Yohimbine is an indolealkylamine alkaloid obtained from *Corynanthe yohimbe* and plants of the *Apocynaceous* family. It blocks certain responses of the effect on cells to epinephrine and sympathetic nerve impulses (Nickerson, 1949); and possesses anti-serotonin activity on peripheral vascular systems (Papesch et al., 1971). High concentrations (10^{-3} g/ml) of yohimbine cause conduction blockage upon repetitive stimulation in frog and toad nerves (Shaw et al., 1955). Lipicky et al.,[1] using voltage clamp techniques, demonstrated that yohimbine reduces the peak sodium current in squid giant axons in a use-dependent manner. Specifically, the extent of the inhibition of sodium current is enhanced by increasing the magnitude of depolarizing pulses and decreased by hyperpolarizing prepulses. These results are interpreted in terms of a modified channel-kinetics model.[1] In that model, it is assumed that yohimbine binds to the gating molecules of sodium channels, and modifies their opening and closing rates. One way to test this hypothesis is to determine the interaction of yohimbine with drugs that are known to bind to the channel gates.

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[1] Lipicky, R. J., D. L. Gilbert, and G. Ehrenstein. Effect of yohimbine on squid axons. Manuscript submitted for publication.

The neurotoxic alkaloids, batrachotoxin (BTX) and veratridine can cause an increase in sodium permeability and depolarization of membrane potential in various nerve and muscle preparations (Narahashi et al., 1971; Albuquerque et al., 1971; Ulbricht, 1969) and in cultured neuroblastoma cells (Catterall and Nirenberg, 1973; Catterall, 1975). Several lines of evidence (considered in the discussion section) suggest that these toxins increase sodium conductance. In these experiments, we have studied yohimbine inhibition of sodium channels activated by batrachotoxin and veratridine. Preliminary results of this work have been reported (Huang et al., 1977).

MATERIALS AND METHODS

Material

Yohimbine was obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.); [^{3}H]leucine, ^{22}NaCl were purchased from New England Nuclear (Boston, Mass.). Veratridine was from K and K Laboratories, Inc., (Plainview, N.Y.). Dulbecco-Vogt modification of Eagle's minimal medium (DMEM) and Dulbecco's phosphate-buffered saline were prepared by the Media Unit of NIH. Fetal calf serum was from Grand Island Biological Co. (Grand Island, N.Y.). Recrystallized trypsin was obtained from Worthington Biochemical Corp.. (Freehold, N.J.). Batrachotoxin was kindly supplied by Dr. J. Daly and Dr. B. Witkop of the Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, NIH. This toxin is stored in ethanol at 100 times the concentration used in the experiment, and was added to the assay media immediately before use.

Cell Culture

Cell line N18 of mouse neuroblastoma C 1300 was used in the present work. The cells were grown as described (Catterall and Nirenberg, 1973). In brief, the cells were propagated in growth medium consisting of 5% fetal calf serum, 95% DMEM in humidified atmosphere of 10% CO_2, 90% air. For experiments, cells were suspended from stock culture with Ca^{++}, Mg^{++}-free Dulbecco's phosphate-buffered saline and 0.02% (wt/vol) trypsin; and were then seeded with growth medium at a density of 15,000 cells/cm^2 in multi-well plates (16 mm diameter, Costar Co., Mass.). The growth medium was changed on days 3 and 5, and cultures were ready for use at day 6 or 7. 0.02 μCi/ml [^{3}H]leucine was added with the growth medium at day 5 so that the protein recovery in the experiments can be measured from ^{3}H counts.

Measurement of ^{22}Na Uptake

For determination of the rate of ^{22}Na uptake, the cells are first preincubated with drug and/or toxin at 36°C for 30 min in a sodium-free medium consisting of 135 mM KCl, 5.5 mM glucose, 0.8 mM $MgSO_4$, and 50 mM Hepes (pH adjusted to 7.4 with Tris). After the cells become equilibrated with the drug, the rate of ^{22}Na uptake is measured for 30 s or the specified time. The uptake medium contains 120 mM choline chloride, 10 mM NaCl, 5.0 mM KCl, 5.5 mM glucose, 0.8 mM $MgSO_4$, 5 mM ouabain, 1.0 μCi/ml ^{22}NaCl. The uptake was terminated by washing the cells four times with 3 ml of 0°C wash medium which is composed of 130 mM choline chloride, 5.0 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM Hepes (pH = 7.4 adjusted with Tris). Under these conditions, the membrane potential of the cell is −41 mV during the $^{22}Na^+$ flux measurement (Catterall, 1977). Since the concentration of Na^+ is low (10 mM), the increase in Na^+ permeability caused by veratridine and batrachotoxin has no effect on membrane potential. The measurements are therefore made at constant membrane potential. Under these conditions, the measured $^{22}Na^+$ influx is directly proportional to sodium permeability ac-

cording to the Goldman-Hodgkin-Katz (1943, 1949) equation. In experiments in which $^{22}Na^+$ influx was measured in the presence of different concentrations of Na^+ and at different membrane potentials, the dependence of measured $^{22}Na^+$ influx on $[Na^+]_{out}$ and on membrane potential conformed to the Goldman-Hodgkin-Katz equation confirming that, under the experimental conditions used, measured $^{22}Na^+$ influx is directly proportional to sodium permeability.

Cells were removed by incubating them in 0.5 ml of 0.4 N NaOH. The suspensions were then transferred into a series of vials, each containing 1 ml of 1 M Tris-HCl (pH = 7.4), and 10 ml of scintillation mixture (5.53% (vol/vol) RPI scintillator fluid, 61.14% toluene and 33.33% Triton X-100). The cell protein is determined by a modification of the Lowry method (Lowry et al., 1951). The uptake is expressed in terms of nanomoles per minute per milligram of protein.

RESULTS

Inhibition by Yohimbine

Batrachotoxin, a toxin produced by the Colombian frog, *Phyllobates aurotaenia,* increases the rate of ^{22}Na uptake in neuroblastoma cells as much as 25-fold (Catterall, 1975). Since this increase is completely abolished by a low concentration of tetrodotoxin (TTX), a specific inhibitor of the sodium channel (Narahashi et al., 1964, Kao, 1966), and is only observable with electrically excitable cells (Catterall and Nirenberg, 1973); the increase in $^{22}Na^+$ uptake is due to activation of sodium channels. The activation of $^{22}Na^+$ uptake reaches half-maximum at 0.4 μM BTX. Most experiments, unless indicated, are performed by using 1 μM BTX, which corresponds to 90% of the maximum response. This concentration is chosen for the present work because of the low solubility of BTX in water and limited supply of the toxin.

We have found that yohimbine blocks the sodium channels in cultured neuroblastoma cells. Specifically, yohimbine inhibits 95% of BTX-dependent activated ^{22}Na uptake at 4 mM concentration (Fig. 1 *a*). This is better illustrated on a semilog plot of the same data vs. time (Fig. 1 *b*). This plot yields a straight line with the slope equal to the time constant k (or $\ln 2/t_{1/2}$ where $t_{1/2}$ is the half time for equilibrium). As shown in Fig. 1 *b*, the increase in the rate of uptake in batrachotoxin solution is almost completely inhibited by 4 mM yohimbine.

The effect of yohimbine concentration on BTX-activated ^{22}Na uptake is given in Fig. 2. The experimental result is fit by a modified Michaelis-Menten equation

$$V = V_{max}\left(1 - \frac{Y}{K_{0.5} + Y}\right) \quad (1)$$

(the solid curve) where $K_{0.5}$ is the apparent dissociation constant of the inhibitor yohimbine, Y the yohimbine concentration, Vmax is the maximum velocity in the absence of inhibitor, V the velocity measured at each yohimbine concentration. The value of $K_{0.5}$ obtained from this dose-response relationship is $1.3 \pm 0.2 \times 10^{-4}$ M.

Inhibition Pattern of Yohimbine

To determine whether yohimbine blocks activation by BTX in a competitive manner, we have measured the ^{22}Na uptake at different concentrations of BTX with various fixed amounts of yohimbine. The data are presented as a Lineweaver-Burk plot

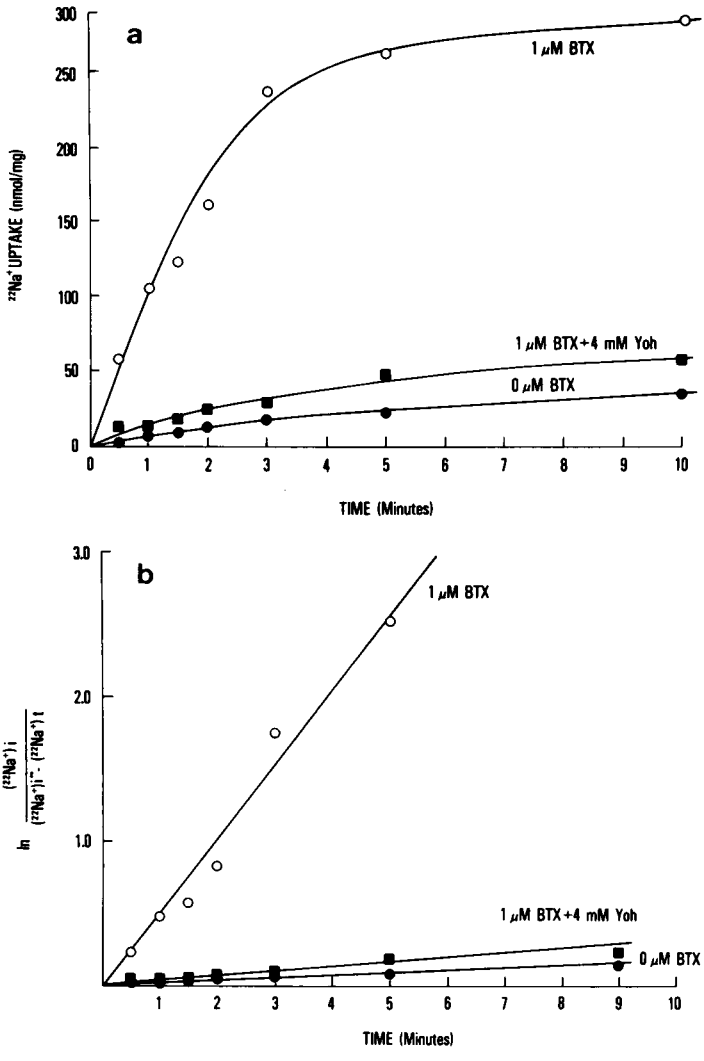

FIGURE 1 (*a*) Inhibition of BTX-dependent ^{22}Na uptake by yohimbine. The cells were first incubated in preincubation media containing no BTX (●), 1 μM BTX (○) or 1 μM BTX plus 4 mM yohimbine (■) for 30 min. The uptake was measured for the time indicated in the uptake medium and the same amount of BTX and yohimbine as in the preincubation medium. (*b*) The same data as in Fig. 1 a are plotted on semilogarithmic coordinates according to the solution of the first order rate equation: $\ln ([Na^+]_i^\infty / ([Na^+]_i^\infty - [Na^+]_i^t)) = kt$.

(Fig. 3). The experimental points fall on straight lines whose slopes vary with yohimbine concentration. Using an iterative least-square procedure, the experimental results were fit to the equation $V = V_{max}B/(K'_{0.5} + B)$ where B is BTX concentration, $K'_{0.5}$ is the "apparent" dissociation constant for BTX, and V_{max} and V have the same definitions as before. Within the limit of experimental accuracy, $K'_{0.5}$ changes markedly with inhibitor concentration, whereas V_{max} is little affected. These results indicate that yohimbine is a competitive inhibitor of BTX activation. Therefore, in Fig. 3, we

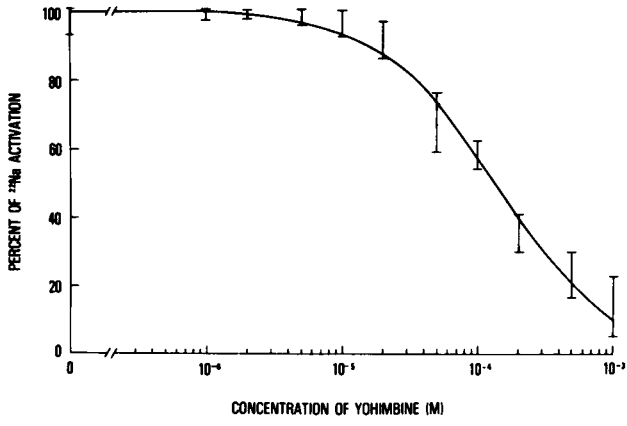

FIGURE 2 Effect of yohimbine concentration on inhibition of BTX-dependent ^{22}Na uptake. Cells were preincubated with 1 μM BTX and the indicated concentration of yohimbine. The uptake was then measured for 30 s in uptake medium consisting of 1 μM BTX and the indicated yohimbine concentration. The Na^+ uptake in cell cultures treated with neither BTX nor yohimbine is subtracted. The ordinate "percent of activation" is the ratio of Na^+ uptake in cells treated with 1 μM BTX and indicated concentration of yohimbine vs. the uptake in cells treated with only 1 μM BTX.

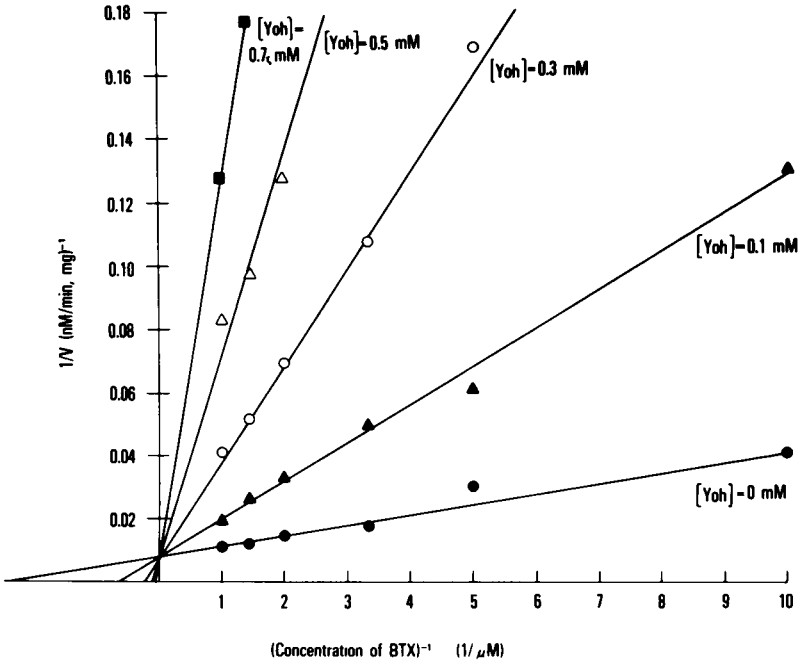

FIGURE 3 Inhibition pattern of BTX-dependent Na^+ uptake by yohimbine. The cells were preincubated with the indicated concentration of BTX containing 0 (●), 10^{-4} M (▲), 3×10^{-4} M (○), 5×10^{-4} M (△), 7×10^{-4} M (■) yohimbine. The solid lines are the least square fits of equation $V\text{max} \cdot B/(K'_{0.5} + B)$ with the assumption of a constant Vmax. The best fit values are Vmax = 130 nmol/min per mg $K'_{0.5}$ = 0.4, 1.6, 4.0, 8.3, 15.6 μM at 0, 10^{-4}, 3×10^{-4}, 5×10^{-4}, 7×10^{-4} M yohimbine.

assumed a constant V_{max} and simultaneously fit all the data at different levels of yohimbine. The best fit values are V_{max} = 130 nmol/min per mg, $K'_{0.5}$ = 0.4 μM at 0 yohimbine, $K'_{0.5}$ = 1.6 μM at 10^{-4} M yohimbine, $K'_{0.5}$ = 4.0 μM at 3×10^{-4} M, $K'_{0.5}$ = 8.3 μM at 5×10^{-4} M and $K'_{0.5}$ = 15.6 μM at 7×10^{-4} M yohimbine. Since yohimbine and batrachotoxin interact competitively, the concentration of yohimbine required for 50% inhibition of Na^+ uptake in the presence of BTX is larger than the true K_D for yohimbine. Calculation of the K_D for yohimbine from the data of Fig. 2 gives a value of 4.1×10^{-5} M.

Veratridine also activates Na^+ channels in neuroblastoma cells (Catterall and Nirenberg, 1973; Catterall, 1975). Experiments similar to those reported above for yohimbine and BTX show that yohimbine also markedly increases the value of $K'_{0.5}$ for veratridine and has little effect on V_{max}. This result is expected since previous experiments showed that veratridine and batrachotoxin act at a common receptor site in activating Na^+ channels (Catterall, 1975).

Rate of Yohimbine Action

The rate of yohimbine action was studied to further substantiate the conclusion that yohimbine, veratridine, and batrachotoxin interact competitively. Cells were first incubated with veratridine or batrachotoxin for 30 min and then were incubated further for various times with yohimbine plus veratridine or batrachotoxin. Finally, $^{22}Na^+$ uptake was measured. The inhibition of veratridine activation was complete in 5 min, whereas the inhibition of batrachotoxin activation required more than 30 min (Fig. 4). This result is inconsistent with a noncompetitive inhibition mechanism in which the nature of the activator should have no effect on the rate of action of the inhibitor. These results are consistent with a competitive inhibition mechanism since the activator must dissociate from its receptor site before the inhibitor can bind. The

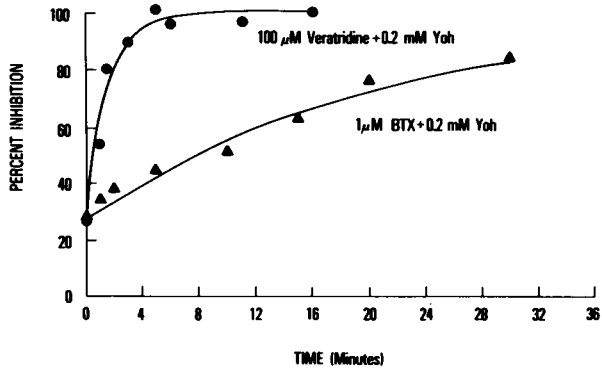

FIGURE 4 Time-course of action of yohimbine. N-18 Cells were first preincubated in Na^+-free preincubation media containing 1 μM BTX for 30 min (▲), or 100 μM veratridine (●) for 5 min. Cells were subsequently incubated in Na^+-free preincubation media with 1 μM BTX plus 2×10^{-4} M yohimbine (▲), or 100 μM veratridine plus 2×10^{-4} M yohimbine (●) for the time shown. Thus the abscissa is the time exposed to yohimbine. The rate of Na^+ uptake was measured in uptake media for 1 min.

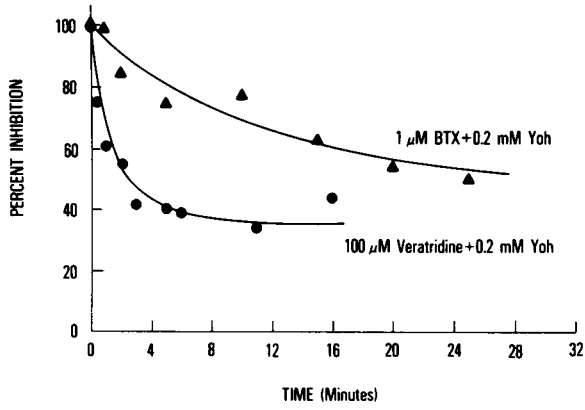

FIGURE 5 The reversal of inhibition by yohimbine. Cells were preincubated in preincubation media containing 1 μM BTX plus 2×10^{-4} M yohimbine (▲), or 100 μM veratridine plus 2×10^{-4} yohimbine (●) for 30 min. After washing the media away, we again incubated the cells in preincubation media with 1 μM BTX (▲) or 100 μM veratridine (●) for the indicated times. The uptakes were assayed in uptake media for 1 min.

rate of yohimbine inhibition of veratridine activation is similar to the rate of dissociation of veratridine ($t_{1/2}$ < 1 min [Catterall, 1975]) and the rate of yohimbine inhibition of batrachotoxin activation is similar to the rate of dissociation of batrachotoxin ($t_{1/2}$ = 30 min [Catterall, 1975]). This is consistent with competitive inhibition if yohimbine binding is relatively fast compared to activator binding.

Experiments in which the rate of recovery from yohimbine inhibition was studied also support a competitive inhibition mechanism (Fig. 5). In these experiments, it is expected from the competitive mechanism that the rates of recovery from yohimbine inhibition will be similar to the rates at which veratridine and batrachotoxin bind to their receptor site. Consistent with this, we find that the rate of recovery in the presence of veratridine is rapid, like the activation of Na^+ channels by veratridine ($t_{1/2}$ < 1 min [Catterall, 1975]), whereas the rate of recovery in the presence of batrachotoxin is slow, like the activation by batrachotoxin ($t_{1/2}$ = 6 min [Catterall, 1975]). If veratridine and batrachotoxin were able to bind to their site of action in the presence of yohimbine, both rates would be rapid reflecting the rapid dissociation rate of yohimbine.[1] Thus both the rate of yohimbine inhibition and the rate of recovery from yohimbine inhibition are determined by the rates of association and dissociation of veratridine and batrachotoxin. These results imply that veratridine and batrachotoxin are excluded from their site of action by yohimbine and thus support a competitive inhibition mechanism.

Inhibition of ^{22}Na Uptake by Yohimbine at Various Membrane Potential

The observations on yohimbine-treated squid axons indicated that the inhibition of sodium current increases with the magnitude of a series of repetitive depolarizing pulses. We have studied the effect of yohimbine at different membrane potentials. From both microelectrode impalement of large neuroblastoma cells and the uptake of

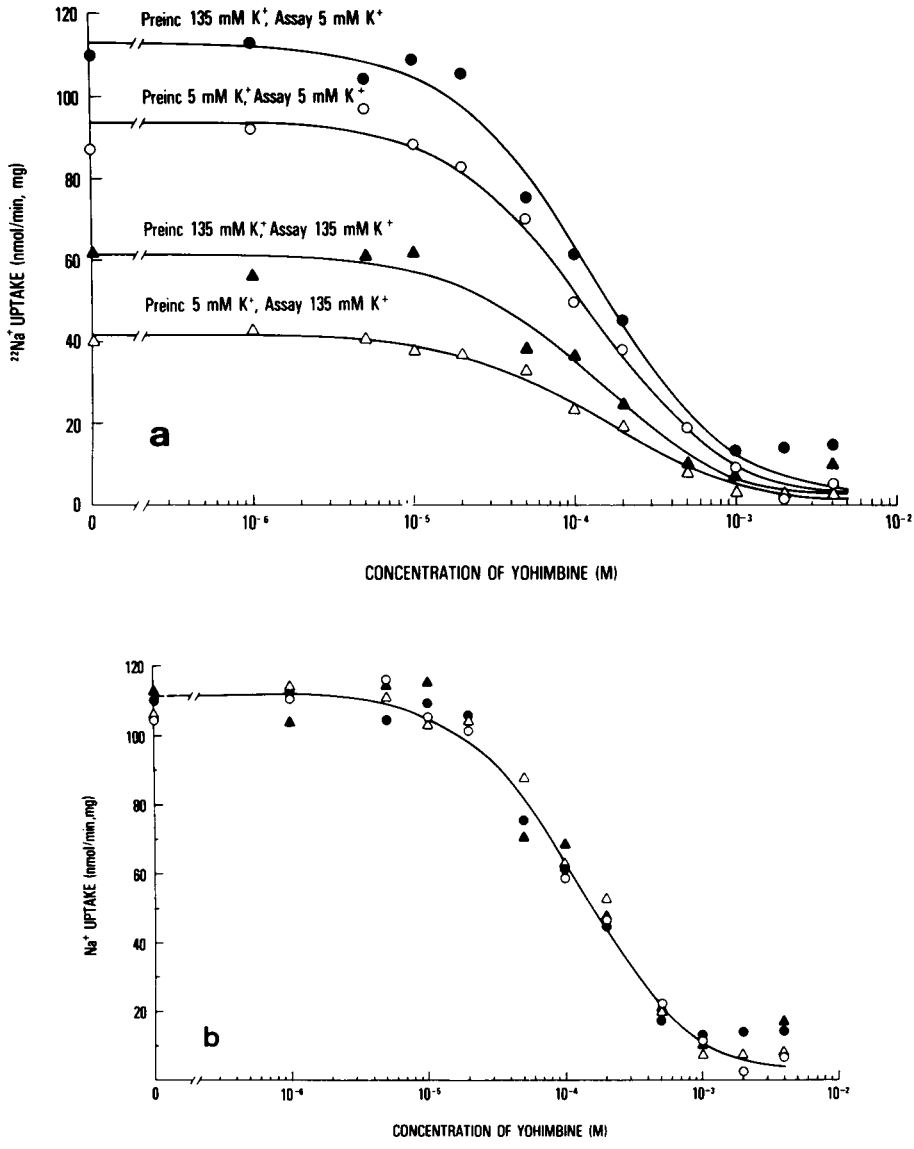

FIGURE 6 (*a*) The effect of membrane potential on inhibition by yohimbine. Cells were preincubated in media containing 1 μM BTX concentration of yohimbine and 135 mM K^+ (●▲) or 5.0 mM K^+ (○△), and choline chloride such that $[K^+] + [\text{choline}^+] = 135$ mM. The rate of uptakes was then measured in assayed media with 135 mM K^+ (▲△), or 5 mM K^+ (●○) and appropriate amount of choline chloride for 30 s. (*b*) The ^{22}Na uptake values measured in different media were normalized with respect to their maximum level of stimulation and fitted by equation $V = V_{max}(1 - Y/(K_{0.5} + Y))$ with $V_{max} = 112.8$ nmol/min per mg and $K = 1.3 \times 10^{-4}$ M.

thiocyanate, the membrane potential was found to be −41 mV at 5.4 mM extracellular potassium, 0 mV at 135 mM potassium, (Catterall et al., 1976). By changing KCl concentration in the preincubation and assay medium, we are able, therefore, to vary the membrane potential in the neuroblastoma cells.

The effect of yohimbine concentration on BTX-dependent uptake at K^+ concentrations of 135 and 5.4 mM, is illustrated in Fig. 6*a*. As shown here, the apparent dissociation constant $K_{0.5}$ does not change as the membrane potential changes from −40 to 0 mV. The lower rate of uptake in $[K]_0$ = 5 mM preincubation medium is due to the loss of intracellular potassium during 30 min of preincubating the cells in a low potassium environment. Thus the ^{22}Na uptake measurement is carried out at somewhat lower membrane potential as compared to those of the cells preincubated in a high K^+ medium. When the ^{22}Na uptake is measured in low and high potassium uptake medium, an identical value of $K_{0.5}$ is obtained. The low rate of uptake in high K^+ uptake medium is again a result of reduction of electrical driving force for inward sodium movement during the uptake period. These ^{22}Na uptake values measured in different media when normalized with respect to their maximum levels of stimulation, can be fitted by a modified Michaelis-Menten equation (Eq. 1) with a single apparent $K_{0.5}$ of $1.3 \pm 0.2 \times 10^{-4}$ M (Fig. 6*b*). Thus, despite the fact that a series of short (5 ms) 40 mV depolarization pulses result in noticeable enhancement of sodium current inhibition by yohimbine in squid, long-term depolarization does not alter the affinity of yohimbine for its binding site in neuroblastoma cells.

DISCUSSION

Batrachotoxin-activated sodium channels have different conductance, gating properties (Khodorov, 1977) and ion selectivity (Khodorov, 1977; Huang et al. 1978) than do normal channels. In comparing our results to the results of voltage clamp experiments on squid axon,[1] it is important to consider whether batrachotoxin acts on normal sodium channels. Several lines of evidence suggest that it does. (*a*) BTX-activated and normal sodium channels are blocked by tetrodotoxin with a K_I in the nanomolar range (Catterall, 1975; Cuervo and Adelman, 1970). In denervated rat muscle and in cultured rat muscle cells, action potentials are relatively resistant to tetrodotoxin. In both of these preparations, high concentration of tetrodotoxin (K_I = 1 μM) is also required to inhibit the sodium permeability increase caused by veratridine and batrachotoxin (Albuquerque and Warnick, 1972; Catterall, 1976). (*b*) In node of Ranvier, Khodorov (1977) has studied the time-course of the action of batrachotoxin. Under the influence of batrachotoxin, the normal sodium current gradually decreases and a new component of sodium current of different kinetics and voltage-dependency gradually develops. (*c*) Variant neuroblastoma cells which specifically lack the depolarizing phase of action potential (Minna et al., 1971) do not respond to treatment with veratridine or batrachotoxin (Catterall and Nirenberg, 1973; Catterall, 1975). These results show that normal sodium channels must be present in order for batrachotoxin or veratridine to increase sodium permeability. These three lines of evidence, taken together, support the conclusion that batrachotoxin or veratridine acti-

vates normal sodium channels. In the discussion to follow, we assume that this is correct.

Batrachotoxin is used to activate the sodium channels in most parts of our studies since batrachotoxin, at 1/200 the concentration of veratridine, yields three to four times larger maximum stimulation of sodium transport activity than veratridine, and thus gives more accurate measurement of Na^+ uptake rates.

Our results show that yohimbine reduces batrachotoxin-induced permeability to sodium. The hyperbolic shapes of the dose-response curve and the reversibility of the inhibition suggest that yohimbine binds to a single class of noninteracting sites with a dissociation constant $K_D = 3\text{–}4 \times 10^{-5}$ M. The rate of action for yohimbine is fast and rate of reversal is also rapid ($t_{1/2} < 1$ min). Most experiments are done by using high potassium (135 mM) preincubation medium and normal potassium (5 mM) uptake medium. This prevents the loss of intracellular potassium during the preincubation period and insures an identical electrochemical driving force for sodium at different yohimbine concentrations during the uptake measurements. The results given here are, therefore, under the condition for the binding of yohimbine in depolarized cells. As shown in Fig. 6, the membrane potential does not change the apparent dissociation constant or the effect of yohimbine. Thus the affinity of yohimbine for its binding site remains constant at different potentials. In a voltage clamp experiment, use-dependent inhibition by yohimbine is observed during depolarizing pulsing.[1] During each pulse, both the membrane potential and the number of open channels change, and it is difficult to establish which effect is the cause of the increased binding of yohimbine. In the experiments reported here, the membrane potential is changed without changing the number of channels, since the number of open channels depends only on the concentration of batrachotoxin. As indicated above, the change of membrane potential does not cause a change in the binding of yohimbine. It follows that membrane potential, itself, is not the cause of use-dependent inhibition. The opening of channels, on the other hand, is a likely cause of the increased inhibition by yohimbine during repetitive pulsing.

The flux experiments shown here are always done in the presence of two drugs, batrachotoxin and yohimbine. This is dictated by the fact that in the absence of an activator such as batrachotoxin, most (>99%) of the Na^+ channels are closed. The difference between the uptake by cells with and without yohimbine treatment would, therefore, be experimentally undetectable. By measuring batrachotoxin-dependent ^{22}Na uptake in the presence of yohimbine, we are able to study the effect of yohimbine on Na^+ channels in neuroblastoma cells. On the other hand, in the electrophysiological studies of squid axon, the yohimbine effect is studied as a reduction in the Na^+ current flow during transient depolarization of the axon. Thus, in the flux experiments, the interaction of two drugs is studied under steady-state conditions whereas, in the voltage clamp experiments, the action of a single drug is studied under transient conditions.[2]

[2]See note 1 above.

Two mechanisms can conceivably account for the reduction of sodium current by an inhibitor. The inhibitor molecule may enter into the mouth of the pore and bind there. The transport of sodium ions is then impeded by the presence of this large molecule in the channel mouth. On the other hand, the inhibitor may bind to a specific site on the gating molecules which may be embedded in the hydrophobic region of the membrane and spatially removed from the pore. The binding changes the free energy for the open and closed states of the gating molecules, and thus affects the sodium permeability.

It has been suggested that tetrodotoxin and saxitoxin block the sodium channels by an occlusion mechanism (Henderson et al., 1974; Hille, 1975). Both of these molecules contain a guanidinium group which is small enough to enter the channels. In contrast, yohimbine is a large hydrophobic molecule and is unlikely to enter the sodium channels. It is probable, therefore, that yohimbine inhibits Na^+ channels by a mechanism that does not involve binding within the ion channel.

Both our kinetic and equilibrium experiments show that yohimbine is a competitive inhibitor of the action of batrachotoxin. These results support the conclusion that yohimbine acts by affecting channel gating processes rather than by occluding the channel. For BTX-activated channels, drugs which bind within the lumen of the ion channel and occlude it must reduce the maximum velocity of ion movement and, therefore, are expected to be noncompetitive inhibitors of the action of batrachotoxin. Tetrodotoxin, which has been postulated to occlude the Na^+ channel (Henderson et al., 1974; Hille, 1975) is a noncompetitive inhibitor of the action of veratridine and batrachotoxin (Catterall, 1975). In contrast, we find that yohimbine is a competitive inhibitor of the action of batrachotoxin and has no effect on the maximum velocity of ion movement at saturation concentration of batrachotoxin. These results clearly distinguish between the sites of action of tetrodotoxin and yohimbine, and indicate that yohimbine does not block the Na^+ channel by occlusion.

The principle alternative mechanism by which yohimbine can inhibit Na^+ current is to reduce the activation of Na^+ channels by altering the gating processes. The competitive inhibition patterns we observe might arise in two ways that are consistent with this mechanism. First, yohimbine may bind at the batrachotoxin receptor site and physically prevent binding of batrachotoxin. Since batrachotoxin alters gating processes (Khodorov, 1977) and activates Na^+ channels, its receptor site must be located on regions of the Na^+ channel involved in gating processes. Interaction of yohimbine with this site would be consistent with its electrophysiologic effects[1] and the competitive interaction demonstrated here. The observed use-dependence of yohimbine inhibition suggests that it binds more rapidly to open Na^+ channels than to closed Na^+ channels.[1] An analogous use-dependent enhancement of batrachotoxin action has been described (Khodorov, 1977). These observations are consistent with the view that yohimbine and batrachotoxin interact with a common site on the gating structures of Na^+ channels.

A second plausible mechanism which also will yield competitive inhibition patterns

is that yohimbine may bind at a site separate from the batrachotoxin receptor site, and reduce the affinity for batrachotoxin.

There are striking similarities in the properties and mechanism of action of drugs like batrachotoxin which activates Na^+ channels, and yohimbine which blocks activation of Na^+ channels. Both drugs are positively charged and lipid soluble. The action of both classes of drugs is enhanced by repetitive activation of Na^+ channels. It seems possible that these two seemingly opposite types of drugs may act via a common mechanism. A unified model for the action of such drugs based on the models presented by Catterall (1975, 1977) and Lipicky et al.[1] is currently under study.

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