

Modulation of Sodium Channel Activity in Frog Ventricular Cells by Guanidyl-side Armed Cyclam

Masuhide YAKEHIRO

Department of Physiology, School of Medicine, Hiroshima University, Hiroshima 734, Japan

ABSTRACT

The mechanism underlying the Na channel blocking action of guanidyl-side armed cyclam (G-cyclam) was studied using conventional patch-clamp methods. G-cyclam applied to the cytoplasmic surface of the membrane reduced the amplitude of single Na channel currents without inducing a flickering block. This effect was enhanced by depolarization and was fully reversible upon washout of the drug. The relationship between the concentration of G-cyclam and the reduction of unitary current could be expressed mathematically assuming one-to-one stoichiometry. During maximal suppression of the single channel current by G-cyclam approximately 40% of the current remained. Low concentration of G-cyclam ($3 \times 10^{-4}M$) prolonged, while higher concentration ($3 \times 10^{-3}M$) shortened the mean open time suggesting the involvement of two processes in the Na channel blocking action of this agent. It appears that low concentration of G-cyclam induce rapid and frequent transition between open and less conductive state resulting in a reduced current, and higher concentration make the channel nonconductive with slower single channel kinetics.

Key words: Sodium channel, Polyamine, Frog ventricular cells, Block

Voltage-clamp recordings have shown that a macrocyclic polyamine with an alkylguanidinium side chain blocks Na channels in squid axons in a voltage- and time- dependent manner, but only when applied to the intracellular membrane surface^{18,19}. We have exploited this pharmacologically unique blocking action of G-cyclam studying the mechanisms underlying Na channel inactivation.

Neher and Steinbach⁵ first showed that the lidocaine derivative QX-222, blocks acetylcholine(ACh) receptors by reducing the mean open time for single channel opening. Thus in the presence of the drug a single channel opening would appear as a burst of much shorter openings. This observation was interpreted to suggest that QX-222 plugs the pores of the ion-conducting pathway through the ACh receptor (occlusion model). A similar hypothesis was postulated by Yamamoto and Yeh²⁴ for a single sodium channel block by 9-aminoacridine. The block of Na channel induced by intracellularly applied tetramethylammonium⁵ and extracellularly applied Ca^{2+} ²⁵ is different to that of 9-aminoacridine since these drugs reduce the single channel current amplitude rather than increase the frequency of transition between open and closed states. However, this effect has been attributed to limitations imposed by the recording system. In addition, many reports examining the effect of local anesthetics and antiarrhythmic drugs on the heart and skeletal muscle^{9,22} proved strong evidence for the occlusion model. Generally, in the case of the open channel block, one can observe either frequent

interruptions of opening event (flickering), leading to the prolongation of burst length, or the prolongation of open time accompanying a reduction in single channel current amplitude.

In this study, we have clarified the mechanism underlying block of Na channels by G-cyclam in frog ventricular cells using the patch-clamp recording technique.

A preliminary account of these experiments has been published elsewhere²³.

MATERIALS AND METHODS

Preparation

The procedure for preparing single ventricular cells from frog, *Rana catesbeiana*, was similar to that described by Seyama and Yamaoka²⁰. However, the following modifications to the original method substantially improved the yield: immediately after dispersion the cells were placed in a low Ca solution for 30 min then following a centrifugation for one min at 600 rpm, the cells were transferred Leibovitz's L-15 medium in which they were stored. These additional procedures were carried out at room temperature.

Solutions

The composition of the solution used for preparing isolated myocytes was as follows; NaCl 93.5 mM, KCl 5.4 mM, $MgSO_4 \cdot 7H_2O$ 5.0 mM, glucose 20 mM, taurine 20 mM, $CaCl_2$ 3.6 μM (pH 7.2 with NaOH) and 5 % fetal calf serum (Gibco, New

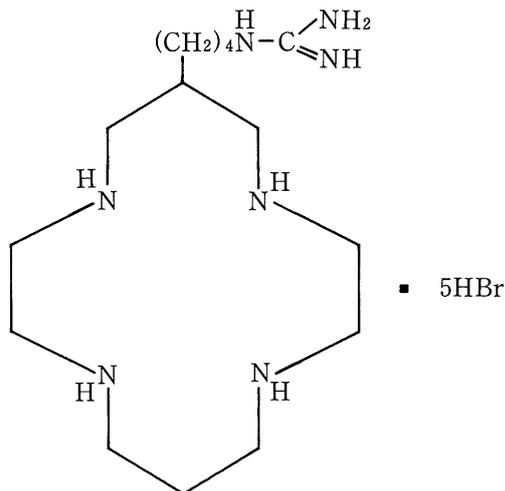


Fig. 1. Structure of G-cyclam.

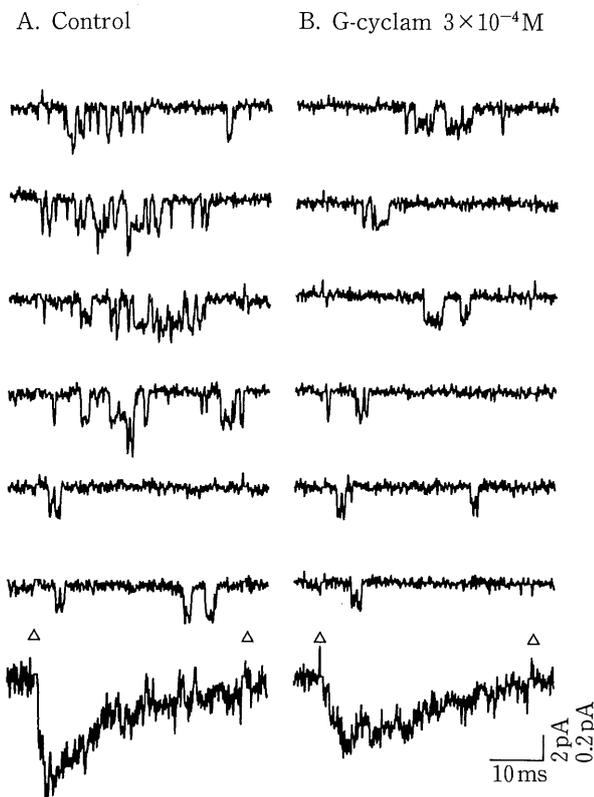


Fig. 2. Single Na channel current recordings from an open cell attached configuration: (A) control and (B) during perfusion of 3×10^{-4} M G-cyclam. The holding potential was -120 mV. The membrane was depolarized to -60 mV for 40 ms (the time bracketed by two open triangles). The ensemble average currents shown at the bottom of both columns were obtained by averaging 100 traces. Temperature was 16°C .

York), penicillin(100 units/ml) and streptomycin(1 mg/ml) was added to Leibovitz's L-15 medium. The bathing (Ringer's) solution consisted of 113.5 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , and 5 mM HEPES (pH 7.2 with NaOH). While the suction

pipette contained 250 mM NaOH, 3 mM MgSO_4 , and 20 mM HEPES (pH 7.2 with aspartic acid). The intracellular solution consisted of 140 mM CsOH, 1mM NaOH, 220 mM sucrose, 5 mM EGTA and 20 mM HEPES (pH 7.0 with aspartic acid).

G-cyclam was a generous gift from Prof. E. Kimura, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine. The stock solution was made by dissolving G-cyclam in distilled water at a concentration of 0.1 M. The test solution containing 10^{-2} M G-cyclam was prepared by dissolving crystalline G-cyclam directly in intracellular solution. The resultant change in pH was corrected using CsOH. The chemical structure of G-cyclam is shown in Fig. 1. Test solutions were prepared immediately before experiments.

Electrical recordings

After formation of a gigaohm-seal and confirmation of Na channel activity in the cell attached configuration, the bathing solution was switched from Ringer's to the intracellular solution. Thereafter single channel current records were recorded using the open-cell attached^{11,4)} or the inside-out membrane patch configuration²⁾. In inside-out membrane patches single channel activity was monitored immediately after application of G-cyclam, while in open cell recordings 10 min were allowed to lapse thus giving the drug time to diffuse to the intracellular surface.

The output of the patch clamp amplifier(L/M-EPC5, LIST, West Germany) was low-pass-filtered at 8 KHz using a Bessel filter(Model E-3201, NF Circuit Design Block Co.Ltd., Yokohama) and digitized at 40 KHz by a digital storage oscilloscope with a 10-bits resolution (KDS-102, Kawasaki Electronics, Tokyo). Digitized records were stored on computer (PC-9801VX, NEC, Tokyo) which also controlled the voltage-clamp protocols used throughout this study.

Capacitive and leakage currents were obtained by fitting a polynomial function to averaged data of records with no openings²¹⁾ and subsequently were subtracted from each original current record. After the subtraction, the current records were further filtered using a digital Gaussian filter¹⁾ at a final cutoff frequency of 3 KHz.

The temperature of the bathing solution was kept constant at 4°C or 16°C , except in the experiments illustrated in Fig. 5 and 7 which were recorded at 24°C (closed triangles).

Analysis method

The transition between conducting and nonconducting states was determined using "half-amplitude threshold analysis"¹⁾. The amplitude of single channel currents was estimated from Gaussian curves fitted to amplitude histograms. While mean open times were determined by fitting single exponential functions to open time histograms

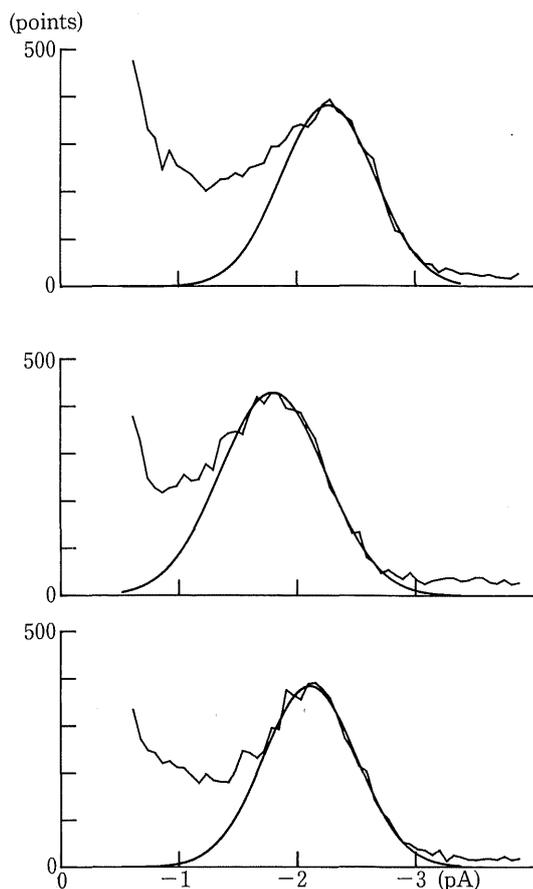


Fig. 3. Amplitude histogram for single Na channel currents before (top), during perfusion of 3×10^{-4} M G-cyclam (middle) and after wash out of the drug (bottom). All records were obtained from the cell shown in Fig. 2. For reasons of clarity the peak representing the closed state has been omitted.

(note, the first two bins were not included in the fit).

RESULTS

Effect of G-cyclam on single channel current

Single Na channel currents in the presence and absence of 3×10^{-4} M G-cyclam in the bathing solution are shown in Fig. 2. In the presence of G-cyclam (Fig. 2B) no apparent flickering of the single channel current was observed. However, there was a marked reduction in the single channel current amplitude, illustrated more clearly by the amplitude histogram shown in Fig. 3. In this example the single channel current amplitude decreased from 2.28 pA in control to 1.82 pA during G-cyclam administration. Removal of G-cyclam caused a complete recovery of the single Na channel current to the control level.

Shown in Fig. 4A is the relationship between single channel current and the patch membrane potential. Under control conditions, the slope conductance was estimated at 15 pS using a least squares fitting routine. 3×10^{-4} M G-cyclam caused a non-linear reduction of the single channel amplitude

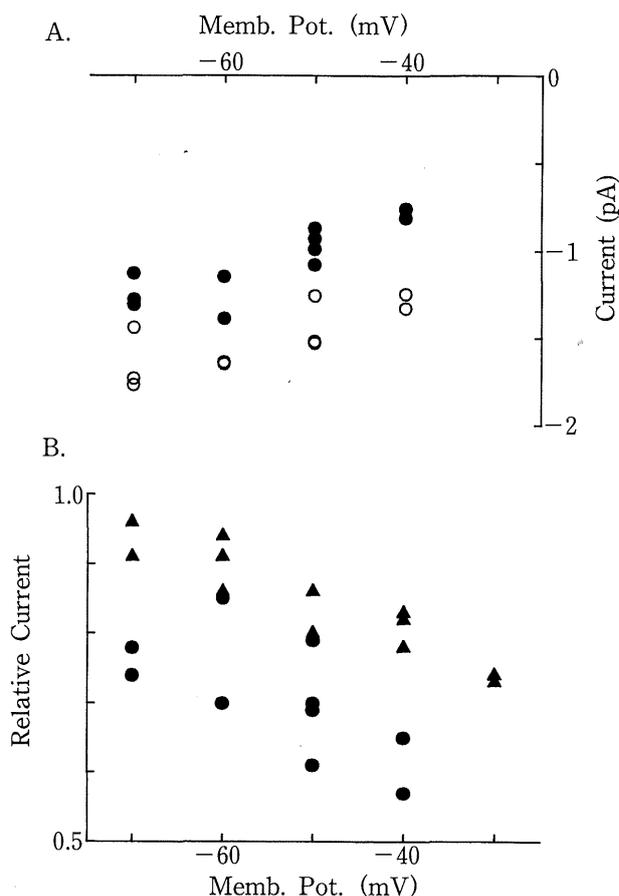


Fig. 4. Relationship between single channel current amplitude and patch membrane potential. (A) Amplitude of the unitary current before (open circle) and during (closed circle) the administration of 3×10^{-4} M G-cyclam at various membrane potentials. (B) Relative amplitude of single Na channel currents during perfusion of 3×10^{-4} M (closed circles) and 1×10^{-4} M (closed triangles) G-cyclam. Ordinate: single Na channel current during drug perfusion relative to control. Abscissa: the membrane potential during test pulse. Single channel currents were recorded using the inside-out membrane patch configuration at a temperature of 4°C .

which increased with depolarization. This effect is more clearly shown in Fig. 4B where the relative amplitudes of single channel currents is plotted against the membrane potential in the presence of 3×10^{-4} M G-cyclam (closed circles) and 1×10^{-4} M G-cyclam (closed triangles). Note, the extent of block is enhanced by depolarization and the increase in G-cyclam concentration.

In order to determine the Q_{10} for the blocking action of G-cyclam, the effect of temperature on mean single channel current amplitude was examined at a range of temperatures in the absence and presence of the drug. The mean single channel current amplitude measured at a membrane potential of -60 mV was 1.54 ± 0.09 pA (mean \pm S.D., $n=5$) at 4°C and 2.20 ± 0.04 pA ($n=5$) at 16°C . The estimated Q_{10} from these values is

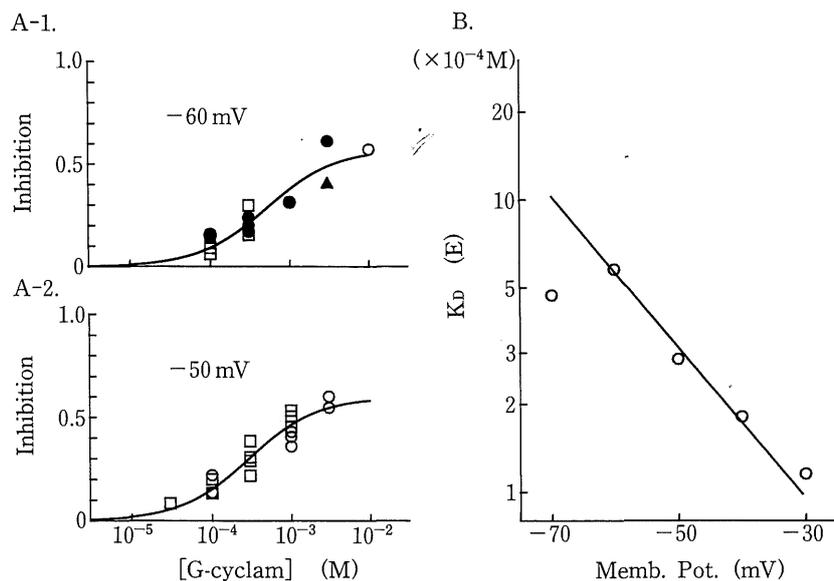


Fig. 5. Concentration-response relationship between G-cyclam and the relative inhibition of single channel current amplitude at -60 mV (A-1) and -50 mV (A-2). Lines were fitted to measured values using a least squares fitting routine, assuming one-to-one stoichiometry. R_{\max} and K_D were calculated to be 0.57 and 5.1×10^{-4} M at -60 mV; and 0.60 and 2.8×10^{-4} M at -50 mV, respectively. Squares indicate data obtained at 4°C , circles at 16°C and triangle at 24°C . The solid triangle, square and circles correspond to those shown in Fig. 7. (B) Voltage-dependency of $K_D(E)$. The solid line fitted using a $K_D(E)$ of $1.62 \times 10^{-5} \cdot \exp(-0.059 \cdot E)$. The value for -70 mV was excluded from calculation, since few single channel events and little effect of G-cyclam was observed at this membrane potential. All data at 16°C and 24°C were recorded in the open cell attached patch configuration while data obtained at 4°C was recorded using the inside-out patch configuration, except on one occasion where the former method was applied (filled square).

1.35. The reduction in single channel current amplitude measured at -60 mV by G-cyclam (3×10^{-4} M) was relatively independent of temperature; relative current is measured to be 0.71 and 0.85 at 4°C and 0.87 , 0.85 and 0.76 at 16°C . The temperature independent blocking action of G-cyclam suggests that access of G-cyclam to its binding site occurs by diffusion perhaps in a manner similar to that of Na ion movement through Na channels³⁾.

Relation between concentration of G-cyclam and the inhibition

The relative inhibition of single channel amplitude (defined as $1 -$ the ratio of single channel amplitude in the presence and absence of G-cyclam) at the membrane potentials of -60 and -50 mV was plotted against the concentration of G-cyclam (Fig. 5, A-1 and A-2). Assuming one-to-one stoichiometry, R_{\max} and $K_D(E)$ were estimated by fitting the data to equation (1).

$$\text{Relative Inhibition} = \frac{R_{\max}}{1 + \frac{K_D(E)}{[\text{G-cyclam}]}} \quad (1)$$

where $K_D(E)$ is the dissociation constant at a membrane potential of E mV, $[\text{G-cyclam}]$ is the concentration of G-cyclam and R_{\max} is the maximum inhibition of single channel current amplitude. A

complete inhibition of Na channel current was not observed at 10^{-2} M or 3×10^{-3} M at membrane potentials of -60 and -50 mV, respectively (Fig. 5, A-1 and A-2). The values of R_{\max} at a membrane potentials of -60 mV and -50 mV were calculated to be 0.57 and 0.60 , respectively. At each membrane potential (from -70 to -30 mV), R_{\max} was less than unity (0.57 , 0.57 , 0.60 , 0.67 and 0.51 for -70 , -60 , -50 , -40 and -30 mV, respectively). The values of $K_D(E)$, assuming an R_{\max} of 0.6 , were calculated and plotted against membrane potential (Fig. 5B). Apparent dissociation constant at 0 mV, $K_D(0)$, was estimated at 1.6×10^{-5} M.

Effect on dwell time

Application of 3×10^{-4} M G-cyclam prolonged the apparent mean open time (determined by fitting a single exponential function to the open time histogram) (Fig. 6A). The ratio for the apparent mean open time during G-cyclam perfusion (τ_d) and the mean open time under control condition (τ_o) was 1.35 ± 0.17 ($n=3$). In contrast, a higher concentration of G-cyclam (3 mM) reduced the ratio of τ_d/τ_o to 0.56 ($n=2$) (Fig. 6B; Fig. 7). A plot of τ_d/τ_o against G-cyclam concentration showed a bell shaped distribution (Fig. 7).

Changes in open probability by drug

In order to determine whether G-cyclam affected the open probability (P_o), cumulative open state

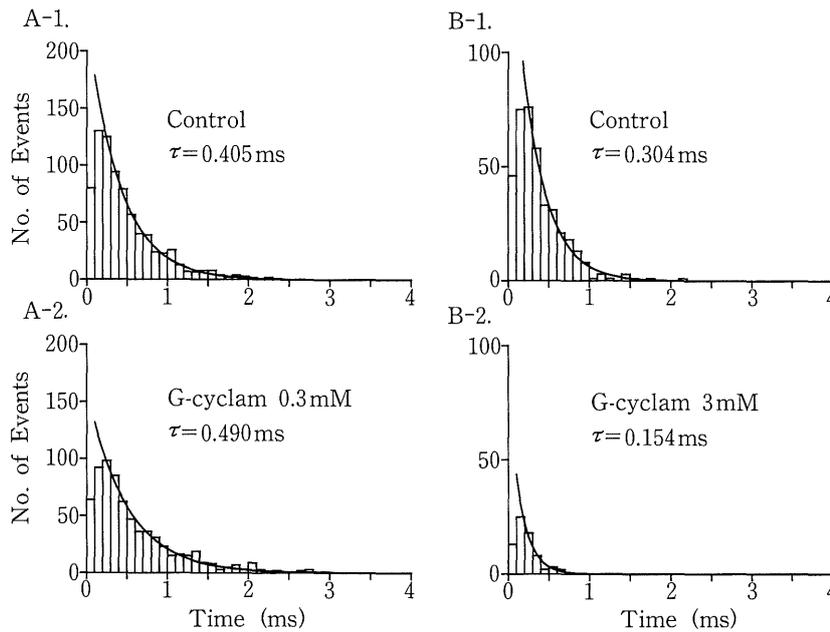


Fig. 6. Open time histogram before (upper panel) and during (lower panel) administration of 3×10^{-4} M (A) and 3×10^{-3} M (B) G-cyclam. Lines were best fitted by a single exponential function. Bin width was 0.1 ms with the first two bins being excluded from the fit. To minimize the error arising from overlapping events and to keep signal to noise ratio high, single channel current records were obtained at a test potential of -60 mV and a temperature of 16°C . Data were recorded using the open cell attached patch configuration.

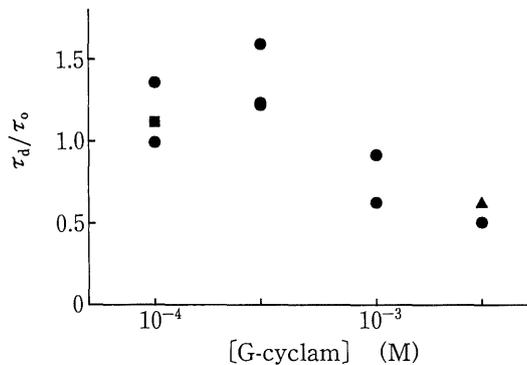


Fig. 7. Relationship between τ_d/τ_0 and G-cyclam. Data were obtained at 4°C (square), 16°C (circles) and 24°C (triangle). Data were recorded using the open cell attached patch configuration.

probability was calculated using the following procedure. First, each single channel current record during a test pulse was averaged and the resultant average current divided by the single channel current amplitude determined from the corresponding amplitude histogram. The values obtained from this calculation which represent the product of the number of channels in a patch and their P_0 was subsequently integrated by the number of traces.

In the simple model given above where G-cyclam can only block open channels, it can be predicted that the open state probability increases as a result of the prolonged mean open time which occurs as the concentration of G-cyclam is increased. Such a change in the open state probability was not observed in the presence of 3×10^{-4} M G-cyclam ($n=3$,

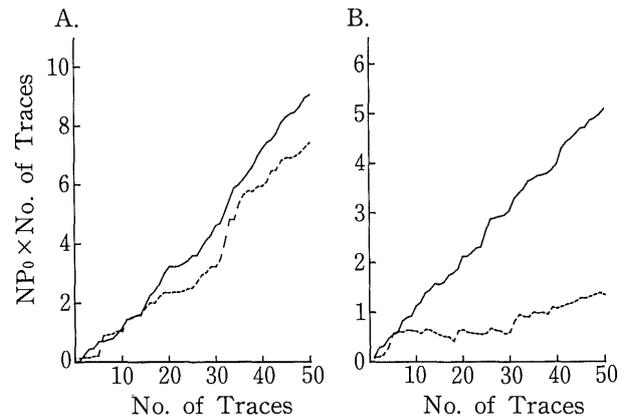


Fig. 8. Cumulative open state probability ($NP_0 \times \text{No. of Traces}$) graphs. Changes in cumulative open probability induced by 3×10^{-4} M and 3×10^{-3} M G-cyclam are shown in graphs A and B, respectively. Solid lines represent the cumulative open probability under control conditions while the dotted lines the cumulative open state probability in the presence of G-cyclam. The number of channels in the patch (N) was 3 in A and 4 in B estimated simply by counting the number of simultaneous channel openings. The membrane potential in these experiments was stepped to -60 mV from holding potential of -120 mV for 40 ms. Data were recorded using open cell attached patch configuration.

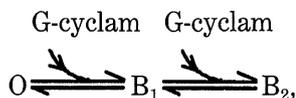
e.g., Fig. 8A). However, contrary to the predictions from the simple model a 10 fold increase in G-cyclam concentration to 3×10^{-3} M significantly decreased the cumulative open probability (Fig. 8B). The effect can be explained as an increase in the

number of null sweep. For example in one experiment there was an increase of null sweeps from 3 to 35 out of a maximum of 50. Furthermore, a shortening of the apparent mean open time was also observed (Fig. 6 B and 7).

DISCUSSION

The results of this study can be summarized as follows. (1) Intracellularly applied G-cyclam reduces the current through single Na channel without inducing a flickering block. (2) The inhibition of unitary Na channel current increases with depolarization and G-cyclam concentration in a fully reversible manner. (3) The concentration-response relationship exhibits Michaelis-Menten type binding with one-to-one stoichiometry. (4) In the presence of G-cyclam the Na channel remains partially conductive with the apparent mean open time being prolonged at concentrations between 10^{-4} and 3×10^{-4} M although higher concentrations (up to 3×10^{-3} M) reduce the apparent mean open time and increase the number of null sweeps indicating a reduction in P_o . These observations partially meet the criteria for simple occlusion model of open Na channels (see Introduction), in which rapid transition between open and blocked states occurs. In support at this notion is the concentration-dependent decrease in apparent single channel current without a noticeable increase of noise and the prolongation in apparent mean open time. However, two observations in this study are incompatible with the simple occlusion model. Firstly, R_{max} in equation (1) for the concentration-response curve is less than 1 (Fig. 5A) and secondly the mean open time decreases at G-cyclam concentrations greater than 3×10^{-4} M (Fig. 7).

These findings can be reconciled with the simple occlusion model using the following kinetic scheme:



where B_1 denotes the first blocked state, equivalent to 40% of the conductance observed in the open state, O, and the nonconductive state B_2 . The closed and inactivated states have been omitted from the model for the sake of simplicity since G-cyclam only blocks open channels^{18,19}. The rate of transition between O and B_1 is very high compared to that of B_1 to B_2 . In this new model, the apparent decrease in single Na channel current in the presence of G-cyclam can be explained by assuming a rapid transition between O and B_1 and, in the presence of high G-cyclam concentrations a decrease of mean open time due to an increase in the rate of transition from B_1 to B_2 . Moreover, the introduction of B_2 may also explain the increase in number of null sweep observed at high concentration of G-cyclam. As the rate constant for

the transition from B_1 to B_2 is also concentration-dependent, sweeps with extremely short channel openings are likely to increase as the concentration of G-cyclam is increased. This could increase the incidence of sweeps containing brief openings which are spuriously categorized as null.

One possible explanation for partial occlusion of Na channels (B_1) is that G-cyclam binds to a site unrelated to the site which binds Na ions and thus affects Na ion translocation. Alternatively, if the channel is viewed as multibarreled structure similar to that proposed for the potential dependent K channel⁶, the inward rectifying K channel⁷ and the Na channel¹⁷, it is possible that each subunit could have a different affinity for G-cyclam. Thus the channel could enter partially conductive states upon occlusion of some of the subunits by G-cyclam.

The alternative explanation for the effects described in this study is simply that G-cyclam binds to the Na channel protein and induces a conformational change (allosteric model). Such a mechanism is thought to be responsible for H^+ block of L-type Ca channels^{12,13,14} and Zn^{2+} block of Na channels¹⁶. In these studies, open channel events were frequently interrupted by a brief, less conductive state ($\sim 1/3$ that of control). The mean dwell time for subconductive states was dependent on ligand concentration. This latter observation cannot be explained using the occlusion model and is consistent with a conformational change in the channel structure. If the blocked time during flickering was measurable in the present study, it could have been possible to discriminate between the occlusion model and the allosteric model.

In summary, block of Na channels by G-cyclam may be explained by two independent processes. The first is due to frequent transition between open and less conductive states with very fast kinetics. This manifests itself as a reduction in single channel conductance. The second, observed at high concentration of G-cyclam is to slow transition from less conductive to nonconductive states. Since Cyclam, which lacks the alkylguanidinium side chain of G-cyclam, but not G-cyclam itself blocks Na currents instantaneously in squid axon, it is likely that the cyclic polyamine part of the molecule is responsible for the fast kinetic component of G-cyclam block and the alkylguanidinium part for the slower component. The importance of the guanidinium group for the closing Na channels has been observed by others. For examples, Rojas and Rudy¹⁵ have reported that alkaline protease b, which cleaves peptide bond containing guanidinium group, prevents Na channel inactivation. Stühmer et al²¹ have demonstrated that the intracellular component between repeats III and IV¹⁰ having several arginines which contain guanidinium group in peptide chain for Na channel is important for the closing mechanism of Na channel by showing no inactivation in the mutant which lacks or cuts DNA for the

component. The speculation raised is in well accord with these experimental results.

ACKNOWLEDGMENTS

I am grateful to Prof. Issei Seyama for his helpful discussions and Prof. Eiichi Kimura for providing G-cyclam. I also thank Mr. H. Miyahara for his assistance in building the experimental apparatus.

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan and a grant from the Tsuchiya Foundation.

(Received July 16, 1992)

(Accepted August 10, 1992)

REFERENCES

1. Colquhoun, D. and Sigworth, F.J. 1983. Fitting and statistical analysis of single-channel records, p.191–263. In B. Sakmann and E. Neher (eds.), *Single-Channel Recording*, Plenum Press, New York.
2. Hamil, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391**: 85–100.
3. Hille, B. 1984. Counting channels, p. 205–225. In *Ionic Channels of Excitable Membranes*, Sinauer, Sunderland.
4. Horie, M., Irisawa, H. and Noma, A. 1987. Voltage-dependent magnesium block of adenosine-triphosphate-sensitive potassium channel in guinea-pig ventricular cells. *J. Physiol. (Lond.)* **387**: 251–272.
5. Horn, R., Patlak, J. and Stevens, C.F. 1981. The effect of tetramethylammonium on single sodium channel currents. *Biophys. J.* **36**: 321–327.
6. Kazachenko, V.N. and Geletyuk, V.I. 1984. The potential-dependent K⁺ channel in molluscan neurons is organized in a cluster of elementary channels. *Biochim. Biophys. Acta* **773**: 132–142.
7. Matsuda, H. 1988. Open-state substructure of inwardly rectifying potassium channels revealed by magnesium block in guinea-pig heart cells. *J. Physiol. (Lond.)* **397**: 237–258.
8. Neher, E. and Steinbach, J.H. 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol.(Lond.)* **277**: 153–176.
9. Nilius, B., Benndorf, K. and Markwardt, F. 1987. Effects of lidocaine on single cardiac sodium channels. *J. Mol. Cell. Cardiol.* **19**: 865–874.
10. Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. and Numa, S. 1984. Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. *Nature* **312**: 121–127.
11. Noma, A. and Tsuboi, N. 1987. Dependence of junctional conductance on proton, calcium and magnesium ions in cardiac paired cells of guinea-pig. *J. Physiol. (Lond.)* **382**: 193–211.
12. Pietrobon, D., Prod'hom, B. and Hess, P. 1988. Conformational changes associated with ion permeation in L-type calcium channels. *Nature* **333**: 373–376.
13. Pietrobon, D., Prod'hom, B. and Hess, P. 1989. Interactions of protons with single open L-type calcium channels. pH dependence of proton-induced current fluctuations with Cs⁺, K⁺, and Na⁺ as permeant ions. *J. Gen. Physiol.* **94**: 1–21.
14. Prod'hom, B., Pietrobon, D. and Hess, P. 1989. Interactions of protons with single open L-type calcium channels. Location of protonation site and dependence of proton-induced current fluctuations on concentration and species of permeant ion. *J. Gen. Physiol.* **94**: 23–42.
15. Rojas, E. and Rudy, B. 1976. Destruction of the sodium conductance inactivation by a specific protease in perfused nerve fibres from *Loligo*. *J. Physiol.(Lond.)* **262**: 501–531.
16. Schild, L., Ravindran, A. and Moczydlowski, E. 1991. Zn²⁺-induced subconductance events in cardiac Na⁺ channels prolonged by batrachotoxin. Current-voltage behavior and single-channel kinetics. *J. Gen. Physiol.* **97**: 117–142.
17. Schreibmayer, W., Tritthart, H.A. and Schindler, H. 1989. The cardiac sodium channel shows a regular substate pattern indicating synchronized activity of several ion pathways instead of one. *Biochim. Biophys. Acta* **986**: 172–186.
18. Seyama, I., Yakehiro, M. and Kimura, E. 1988. Dynamics of guanidyl-side armed cyclam (G-cyclam) and cyclam block of Na channels in squid giant axons. *Biomedical Research* **9**: S89–90.
19. Seyama, I., Yakehiro, M., Yamaoka, K., Nakajima, T. and Kimura, E. 1986. Mechanism of the Na channel block with trypargine and guanidyl-side armed cyclam, p. 101–109. In H. Imura, T. Goto, T. Murachi and T. Nakajima (eds.), *Natural Products and Biological Activities: A Naito Foundation Symposium*, University of Tokyo Press, Tokyo.
20. Seyama, I. and Yamaoka, K. 1988. A study of the electrical characteristics of sodium currents in single ventricular cells of the frog. *J. Physiol.(Lond.)* **401**: 257–275.
21. Stühmer, W., Conti, F., Suzuki, H., Wang, X., Noda, M., Yahagi, N., Kubo, H. and Numa, S. 1989. Structural parts involved in activation and inactivation of the sodium channel. *Nature(Lond.)* **339**: 597–603.
22. Wang, G. K. 1988. Cocaine-induced closures of single batrachotoxin-activated Na⁺ channels in planar lipid bilayers. *J. Gen. Physiol.* **92**: 747–765.
23. Yakehiro, M. and Seyama, I. 1990. Interaction of guanidyl-side armed cyclam (G-cyclam) with single sodium channels in frog ventricular cells. *Jpn. J. Physiol.* **40**: S260.
24. Yamamoto, D. and Yeh, J.Z. 1984. Kinetics of 9-aminoacridine block of single Na channels. *J. Gen. Physiol.* **84**: 361–377.
25. Yamamoto, D., Yeh, J.Z. and Narahashi, T. 1984. Voltage-dependent calcium block of normal and tetramethrin-modified single sodium channels. *Biophys. J.* **45**: 337–344.