0026-895X/90/050720-05$02.00/0

MOLECULAR PHARMACOLOGY, 37:720-724

Niflumic and Flufenamic Acids are Potent Reversible Blockers of Ca^{2+}-Activated Cl^- Channels in *Xenopus* Oocytes

MICHAEL M. WHITE and MARIA AYLWIN

Departments of Pharmacology (M.M.W.) and Physiology (M.A.), University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received August 17, 1989; Accepted January 4, 1990

SUMMARY

The effects of niflumic acid and flufenamic acid, two nonsteroidal anti-inflammatory agents known to block anion transport in red blood cells, on Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) in *Xenopus* oocytes were examined. Both compounds reversibly inhibited $I_{Cl(Ca)}$, elicited in response to depolarizing voltage steps, in a dose-dependent manner, with no effect on the shape of the current-voltage curve. The apparent inhibition constant for niflumic acid was 17 μM, whereas that for flufenamic acid was 28 μM. Niflumic acid also inhibited $I_{Cl(Ca)}$ elicited by bath application of Ca^{2+} to oocytes permeabilized using the Ca^{2+} ionophore A23187, demonstrating that the inhibition of $I_{Cl(Ca)}$ is due to a direct interaction with the Cl^- channel, rather than by interference with Ca^{2+} entry through voltage-dependent Ca^{2+} channels. In addition to their use in the elimination of $I_{Cl(Ca)}$ as a possible source of artifact when *Xenopus* oocytes are used as an expression system for exogenous ion channels and receptors, it is expected that these two compounds will find use as potent anion channel blockers.

Xenopus oocytes have proven to be a useful tool in the study of the molecular properties of ion channels and receptors (for a review see Ref. 1). This is due to the fact that 1) they can synthesize and assemble a variety of ion channels and receptors after the injection of RNA either isolated from various tissues (2) or synthesized *in vitro* from cDNA clones (3, 4) and 2) they are amenable to a variety of electrophysiological (5) and biochemical (6, 7) techniques. An additional advantage of the oocyte expression system is that *Xenopus* oocytes contain only a small number of endogenous ion channels, among them a delayed rectifier K^+ current (8), a transient Ca^{2+} current (9), and an outward transient Ca^{2+}-activated Cl^- current ($I_{Cl(Ca)}$) (8, 10). The presence of the first two endogenous voltage-dependent channels does not usually interfere with the recording of currents encoded for by exogenous RNAs, because the total current carried by them is quite small relative to those from exogenous channels. However, the presence of $I_{Cl(Ca)}$ can cause complications. For example, an inward Ca^{2+} current of 10 nA can produce a transient outward Cl^- current of greater than 100 nA, thus masking the Ca^{2+} current. In order to measure current flowing through either endogenous or exogenous Ca^{2+} channels, Dascal *et al.* (9) had to use recording solutions in which the Cl^- had been replaced by methanesulfonate and the Ca^{2+} replaced by high concentrations of Ba^{2+} [which is relatively ineffective at activating $I_{Cl(Ca)}$ (11)].

Although one expects that activation of Ca^{2+} channels will lead to the activation of $I_{Cl(Ca)}$ and thus uses the appropriate saline solutions to observe Ca^{2+} currents, there are other instances when $I_{Cl(Ca)}$ unexpectedly appears. Any manipulation that leads to the elevation of intracellular Ca^{2+} will cause the Cl^- channels to open and, thus, produce either an inward or outward current, depending on the driving force for Cl^- movement. For example, Mishina *et al.* (4) found that, when *Torpedo* AChR were expressed in *Xenopus* oocytes using RNA trancribed *in vitro* from cDNA clones, the levels of AChR expression was such that enough Ca^{2+} entered through the AChR channels to activate $I_{Cl(Ca)}$, leading to a biphasic current response to iontophoretic application of ACh, with the latter phase being due to activation of $I_{Cl(Ca)}$. These workers resorted to the use of Ca^{2+}-free saline solutions for studies involving the use of macroscopic current measurements. However, desensitization of AChRs requires extracellular Ca^{2+} (12), so this manipulation may not be a satisfactory solution to the problem, and one is usually limited to working at the Cl^- reversal potential (−25 mV) if one wishes to study AChR desensitization (13). More recently, Kelso and Leonard (14) have suggested that the apparent desensitization of NMDA receptor-mediated currents expressed in oocytes may be an artifact of Ca^{2+} entry through the NMDA receptor channel, with the early transient phase of

This work was supported by National Institutes of Health Grant NS 23885 and Alfred P. Sloan Research Fellowship BR-2733.

ABBREVIATIONS: $I_{Cl(Ca)}$, Ca^{2+}-activated Cl^- current; AChR, acetylcholine receptor; NMDA, *N*-methyl-D-aspartate; DIDS, 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene; SITS, 4-acetamino-4′-isothiocyano-2,2′-disulfonic acid stilbene; 9-AC, anthracene-9-carboxylic acid; NFA, niflumic acid; FFA, flufenamic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GABA, γ-aminobutyric acid; ACh, acetylcholine; EGTA, ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

the NMDA-elicited currents actually being due to activation of $I_{Cl(Ca)}$.

Most, if not all, of these problems could be avoided if there existed a fairly potent blocker of $I_{Cl(Ca)}$ in *Xenopus* oocytes that was without effect on other channels and receptors. Although numerous blockers of various cation channels exist (15), there are relatively few anion channel blockers. The stilbene disulfonates such as DIDS and SITS are potent irreversible blockers of both anion transport systems (16) and anion channels (17, 18). However, DIDS and SITS are somewhat nonspecific and can covalently interact with other membrane proteins, including K^+ channels (19), and thus are somewhat undesirable as general purpose anion channel blockers. 9-AC is a potent reversible blocker of skeletal muscle Cl^- channels ($K_d = 11\ \mu M$) (20), but it has a much lower affinity for $I_{Cl(Ca)}$ in oocytes ($K_d = 110\ \mu M$)[1] and one must use high concentrations of 9-AC (≥1 mM) to get essentially complete block of $I_{Cl(Ca)}$ in oocytes. In addition, 9-AC is a potential carcinogen and, thus, is clearly not the compound of first choice.

The nonsteroidal anti-inflammatory agent NFA is a potent noncompetitive inhibitor of the red cell anion transporter, with an inhibition constant of approximately 1 μM (21, 22). Brule *et al.* (23) reported that NFA inhibited inwardly rectifying Cl^- currents in crayfish muscle. We report here that both this compound and a related nonsteroidal anti-inflammatory agent, FFA, reversibly block $I_{Cl(Ca)}$ in *Xenopus* oocytes, with inhibition constants of 17 and 28 μM, respectively. We expect that these compounds will prove to be useful tools in electrophysiological studies involving the expression of exogenous ion channels in *Xenopus* oocytes.

Materials and Methods

Isolation of *Xenopus* oocytes. Mature female *Xenopus* were obtained from Xenopus One (Ann Arbor, MI) and maintained in the laboratory until used. A frog was anesthetized by immersion in water containing 0.17% tricaine. A small incision was made in the abdomen and several ovarian lobes were removed and placed in physiological saline (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6) supplemented with 50 μg/ml gentamycin sulfate. The surrounding follicle cell layer was removed by incubation of the tissue in Ca^{2+}-free OR-2 (82 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6) containing 2 mg/ml collagenase (Type IA, Sigma), for 2 hr at 19°. The oocytes were transferred to the antibiotic-supplemented saline and the adhering follicular cell layer was removed with forceps. Isolated stage V and VI oocytes (24) were transferred to saline supplemented with gentamycin and 2.5 mM sodium pyruvate. Oocytes were maintained in this solution at 19° for several days after isolation for recording.

Expression of exogenous receptors. Total RNA was isolated from the brains of 12-day-old rats, using the urea/LiCl method of Auffray and Rougeon (25), and was stored at −70° in distilled water at a concentration of 4 mg/ml. Oocytes were microinjected as previously described (3), with the exception that 200 ng of RNA were injected per oocyte. Injected oocytes were maintained as described above, and recordings were made 2–3 days after injection.

Electrophysiology. Individual oocytes were transferred to the recording chamber and studied under voltage-clamp conditions, using a standard two-microelectrode voltage clamp (Axoclamp 2A; Axon Instruments, Foster City, CA). Electrodes were filled with 3 M KCl and had resistances of 0.5–5 MΩ. The chamber was continually perfused with high-Ca^{2+} saline (96 mM NaCl, 2 mM KCl, 10 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6). All voltage steps were made from a holding potential of −100 mV. This holding potential was chosen in order to remove resting inactivation of the oocyte Ca^{2+} channels, and the high extracellular Ca^{2+} was chosen to further enhance $I_{Cl(Ca)}$ by increasing Ca^{2+} entry. Pulse generation and data acquisition were under control of a DEC 11/73-based data acquisition system (Indec Systems, Sunnyvale, CA). Each set of currents recorded in the presence of the desired concentration of the compound under study was preceded and followed by a set of currents recorded in the absence of the drug, in order to control for any run-down of the currents during the course of the experiment.

For experiments involving permeabilized oocytes, the procedure of Boton *et al.* (26) was used. Briefly, oocytes were incubated for 10 min in Ca^{2+}-free OR-2 supplemented with 20 mM $MgCl_2$ and 0.5 mM EGTA and then for another 15 min in the same solution containing 1 μM A23187. The oocyte was then transferred to the recording chamber and

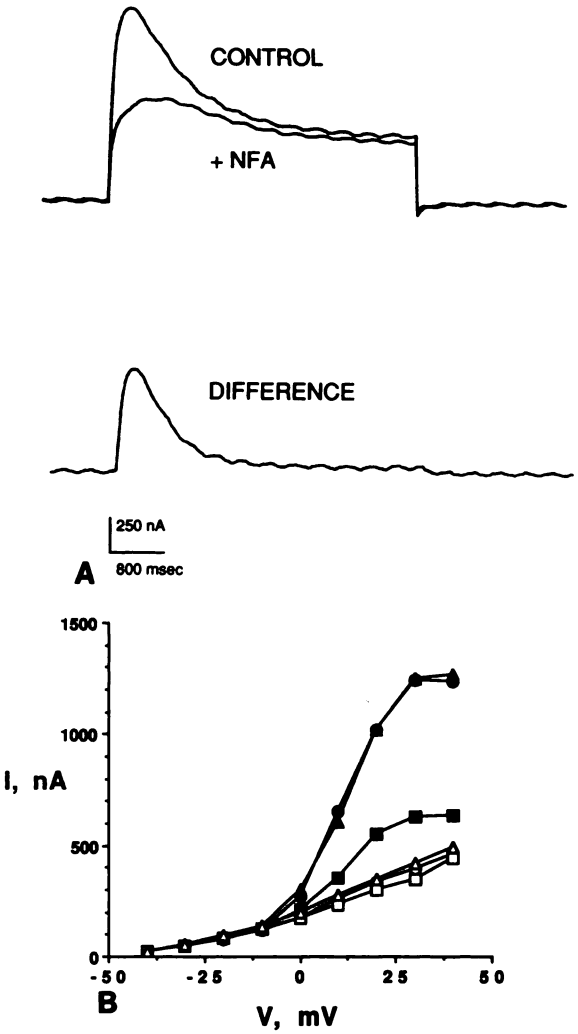

Fig. 1. A, NFA blocks Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes. An oocyte was voltage clamped as described in Materials and Methods. Currents elicited in response to a 4.5-sec voltage step from −100 mV to +30 mV were recorded in the absence or in the presence of 50 μM NFA. The *lower trace* shows the difference current, which is the current blocked by NFA. Note that the transient current is reduced in the presence of NFA, whereas the late steady state current is not. B, NFA block of $I_{Cl(Ca)}$ is reversible and voltage independent. A series of 4.5-sec voltage steps from a holding potential of −100 mV to the indicated potential were applied to an oocyte in the absence of NFA (*circles*), in the presence of 50 μM NFA (*squares*), and 2 min after NFA was washed out (*triangles*). *Solid symbols*, total peak current level; *open symbols*, steady state levels. Note that the NFA block is reversible, that the shape of the *I-V* curve is unchanged in the presence of NFA, and that the steady state current is essentially unaffected by NFA.

[1] M. M. White, unpublished data.

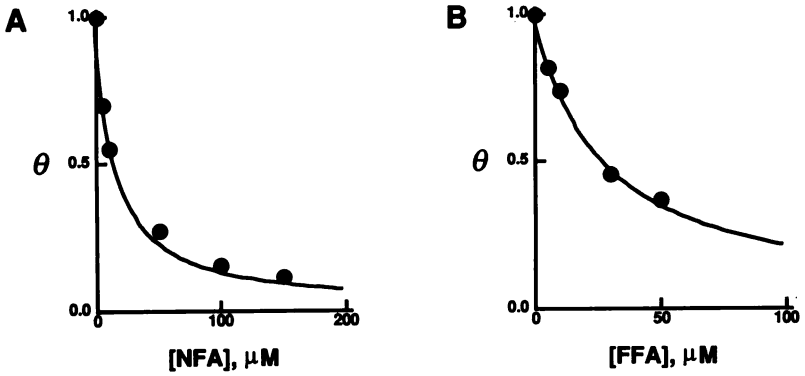

Fig. 2. A, Dose dependence of NFA block of $I_{Cl(Ca)}$. $I_{Cl(Ca)}$ at +30 mV (defined as peak current minus the steady state current) was measured in the presence of 0–150 μM NFA. The fractional current, θ, was determined as described in the text and is plotted in A. The *smooth line* is the fit using an inhibition constant, K_I, of 16 μM. These data were obtained from a single oocyte. The value of K_I from a series of determinations was 17 ± 1 μM (mean ± SE; n = 8). B, Dose dependence of FFA block of $I_{Cl(Ca)}$. Conditions were as in A, with the exception that 0–50 μM FFA was used. The data are taken from a single oocyte, and the value of K_I obtained from the analysis was 27 μM. The value of K_I from a series of determinations was 28 ± 2 μM (mean ± SE; n = 4).

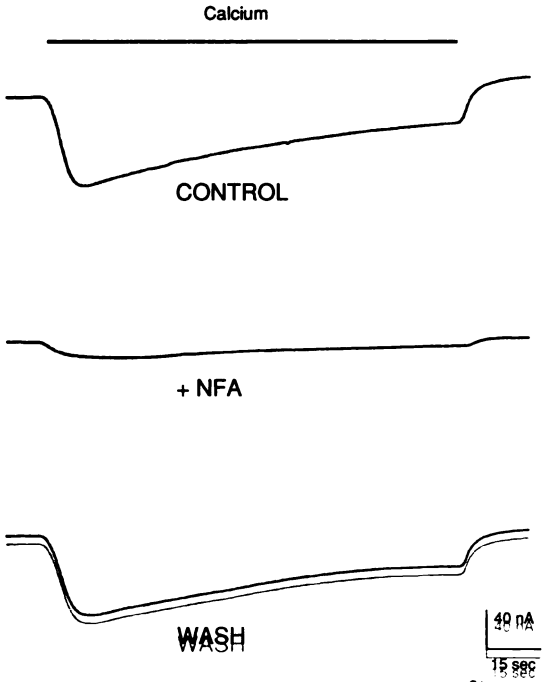

Fig. 3. NFA blocks $I_{Cl(Ca)}$ evoked by bath application of Ca^{2+} to permeabilized cells. An oocyte was Ca^{2+} permeabilized using A23187, as described in Materials and Methods. The oocyte was voltage clamped at −60 mV in Ca^{2+}-free saline. $I_{Cl(Ca)}$ was evoked by switching the perfusate to a solution containing 1 mM Ca^{2+}, in the absence of NFA, in the presence of 50 μM NFA, and 2 min after NFA was washed out. In the *middle trace*, NFA was added 60 sec before application of Ca^{2+} and then removed 60 sec after washout of Ca^{2+}. Note the reversible inhibition of $I_{Cl(Ca)}$ by NFA.

perfused with Ca^{2+}-free OR-2 with 20 mM $MgCl_2$ and 0.5 mM EGTA for 10 min and then with the same solution without EGTA. The oocyte was then voltage clamped at −50 mV and $I_{Cl(Ca)}$ was evoked by perfusing the chamber with the OR-2 containing 20 mM $MgCl_2$ and 1 mM $CaCl_2$.

Data analysis. Inhibition constants for NFA and FFA were determined by fitting the data to a single-site titration model:

$$\theta(c) \equiv (1 \pm ([c]/K_I))^{-1} \quad (1)$$

where $\theta(c)$ is $I_{Cl(Ca)}$ in the presence of the blocker at concentration c, normalized to that in the absence of the blocker, and K_I is the inhibition constant for the drug. The steady state current level was subtracted from the peak current values in order to define $I_{Cl(Ca)}$. Fits were obtained using a Levenberg-Marquart algorithm in a commercially available Apple Macintosh-based analysis program (IGOR; WaveMetrics, Lake Oswego, OR).

Materials. NFA and FFA were obtained from Sigma and stored at 150 mM stock solutions in absolute ethanol. Ethanol at a concentration of 0.1% had no effect on $I_{Cl(Ca)}$. A23187 was obtained from Calbiochem and stored as a 100 mM stock solution in dimethyl sulfoxide.

Results

Depolarization of voltage-clamped *Xenopus* oocytes elicits a transient outward current varied by Cl^- ions evoked by the entry of Ca^{2+} through voltage-dependent Ca^{2+} channels (8, 10). This current can be enhanced by increasing the extracellular Ca^{2+} concentration and using a holding potential that removes resting inactivation of the Ca^{2+} channels. Fig. 1A shows the membrane current elicited by a 4.5-sec voltage step from −100 mV to +30 mV. A transient outward current of approximately 1.5 μA is clearly visible, as is a steady state current of approximately 300 nA. The figure also shows current elicited in the same cell in response to the same voltage step in the presence of 50 μM NFA. Note that, although the transient current is diminished in the presence of NFA, the maintained steady state current is essentially unaffected by the presence of NFA, which suggests that NFA blocks only the transient outward current carried by the Ca^{2+}-activated Cl^- channels $I_{Cl(Ca)}$ but not the maintained outward current, which is not carried by Cl^- (10). The lower part of the figure shows the difference current, which shows that the current blocked by NFA consists of a transient current, as expected if NFA blocked only $I_{Cl(Ca)}$.

Fig. 1B shows the voltage dependence of the peak and steady state current before NFA, in the presence of 50 μM NFA, and after washing out of the drug. In all three cases, the maximum of the current-voltage relationship occurs at +30 to +40 mV. In addition, the overall shape of the current-voltage relationship is unaffected by the presence of NFA, indicating that NFA does not perturb the gating process of the channels. Finally, the figure also shows that the current-voltage relationship after the NFA is washed out is identical to that taken before application of NFA, showing that the effect of NFA is completely reversible. NFA had essentially no effect on the steady state current at the end of the pulse, throughout the entire voltage range.

Fig. 2 shows the concentration dependence of the block of $I_{Cl(Ca)}$ (defined as the peak current minus the steady state current) by NFA and FFA at +30 mV. The data are fit to a single-site inhibition model:

$$\theta(c) \equiv (1 \pm ([c]/K_I))^{-1} \quad (1)$$

where $\theta(c)$ is $I_{Cl(Ca)}$ in the presence of the blocker at a concentration c, normalized to that in the absence of the blocker, and K_I is the inhibition constant. The values of K_I obtained from the data in Fig. 2 are 16 μM for NFA and 27 μM for FFA; from

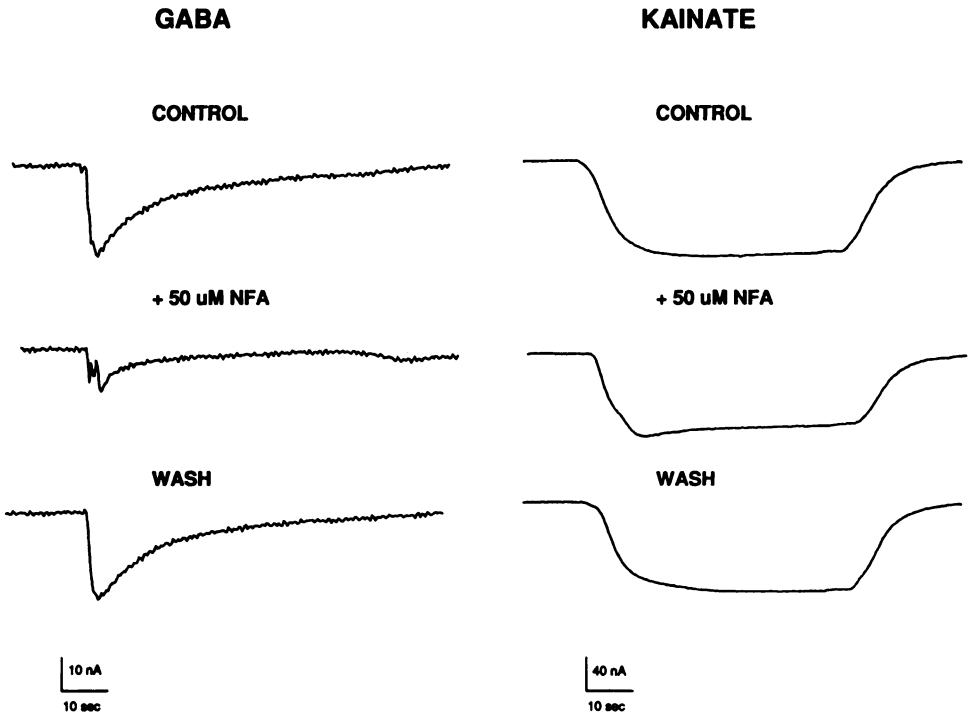

Fig. 4. NFA blocks GABA-elicited Cl^- currents but not kainic acid-elicited cation currents. Oocytes were microinjected with rat brain RNA, as described in Materials and Methods. Oocytes were voltage clamped at −60 mV in normal saline and the current responses to a 1-min application of either 500 μM GABA (*left*) or 100 μM kainic acid (*right*) were measured. Responses obtained before, during, and after exposure to 50 μM NFA are shown. NFA was added 60 sec before application of agonist and removed 60 sec after agonist washout.

a series of experiments, the values are 17 ± 1 μM (mean ± SE of eight determinations) for NFA and 28 ± 2 μM (mean ± SE of four determinations) for FFA.

The data in Figs. 1 and 2 suggest that NFA and FFA block Ca^{2+}-activated Cl^- channels. However, in addition to directly blocking the Cl^- channel itself, one can obtain a reduction in $I_{Cl(Ca)}$ by reducing the entry of Ca^{2+} through the voltage-dependent Ca^{2+} channels. One way to distinguish between these two possibilities is to examine the effect of these blockers on $I_{Cl(Ca)}$ elicited by increasing intracellular Ca^{2+} by a mechanism independent of the Ca^{2+} channels. Ionophore A23187 can be used to elevate intracellular Ca^{2+} by bath application of Ca^{2+} to oocytes previously treated with the ionophore in the absence of Ca^{2+} (26). Fig. 3 shows $I_{Cl(Ca)}$ elicited at −60 mV by bath application of 1 mM Ca^{2+}. A slowly decaying inward Cl^- current is elicited by changing the perfusate from a Ca^{2+}-free solution to a Ca^{2+}-containing solution, and the current rapidly returns to baseline when the perfusate is returned to the Ca^{2+}-free solution. When 50 μM NFA is present in the perfusate, the response to Ca^{2+} is reduced and, when the NFA is washed out, the response to Ca^{2+} application is restored to that seen before the application of NFA. These data indicate that NFA reduces $I_{Cl(Ca)}$ by interacting with the Cl^- channel directly.

In order to further examine the specificity of these two compounds, GABA and kainic acid receptors were expressed in *Xenopus* oocytes by injection of rat brain RNA. Cl^- currents elicited by bath application of 500 μM GABA were reduced to 40% of control level by 50 μM NFA, whereas inward cation currents elicited by the application of 100 μM kainic acid were essentially unaffected by 50 μM NFA (Fig. 4). These results, when taken with the other results presented here, suggest that NFA and FFA may be fairly broad spectrum anion channel-specific channel blockers.

Discussion

A common problem in the analysis of electrophysiological responses is the fact that a given response may contain contributions from currents other than the one of interest. The judicious use of appropriate stimuli in conjunction with the proper recording solution and channel blockers makes it possible in many cases to isolate the current of interest. Although there are many different blockers for various cation channels, there are relatively few potent broad spectrum anion channel-specific blockers. Thiocyanate and iodide have been shown to block the voltage-dependent Cl^- channel from *Torpedo* electroplax (17), but these two anions can permeate a number of other anion-selective channels (27). The stilbene disulfonates DIDS and SITS suffer from the drawback that they can also exhibit reactivity towards other channels and, thus, may introduce undesired side effects. Finally, 9-AC, although exhibiting high potency towards the skeletal muscle Cl^- channel (20), is much less effective in inhibiting other anion-selective channels including $I_{Cl(Ca)}$ channels (28), and this may hold true for other anion channels as well. Thus, none of these commonly used agents fits the bill as widely applicable potent anion channel blockers.

NFA is a potent inhibitor of the red blood cell anion transporter (21, 22). Although there is no *a priori* reason to expect that a compound that inhibits a transporter will also block a channel, this compound was found to inhibit inward rectifier Cl^- currents in crayfish muscle, with half-maximal inhibition seen at a concentration of approximately 1 μM (23). More recently, Evoniuk and Skolnick (29) showed that NFA blocked the anion modulation of the binding of *t*-butylbicyclophosphorothioate to the GABA receptor, with IC_{50} values ranging from 30 to 155 μM. This anion binding site is thought to be somehow related to the anion permeation pathway in the GABA receptor complex, and these data suggest that NFA may block the GABA receptor channel. The data presented in Fig. 4A show that NFA does indeed block GABA-evoked Cl^- currents.

The data presented in this paper clearly demonstrate that both NFA and the related compound FFA potently and revers-

ibly block endogenous Ca^{2+}-dependent Cl^- channels in *Xenopus* oocytes, with inhibition constants of 17 ± 1 μM and 28 ± 2 μM, respectively. NFA also blocks GABA-elicited Cl^- currents expressed in oocytes (Fig. 4). In addition, FFA also reversibly blocks the voltage-dependent Cl^- channel from *Torpedo* electroplax (17), with an inhibition constant of 28 μM.[1] Finally, these compounds are ineffective at blocking either the steady state outward current in oocytes (Fig. 1) or kainic acid-elicited cation currents expressed in oocytes (Fig. 4). Thus, these two compounds seem to block a number of different anion channels with fairly high affinity and may prove to be quite useful as anion channel blockers. From our own point of view, we expect these two compounds to be particularly useful for those who work on the expression of ion channels and receptors in *Xenopus* oocytes, because the presence of Ca^{2+}-activated Cl^- channels in these cells can introduce unexpected and unsuspected compounds in recorded currents that could be potentially misinterpreted.

References

1. Snutch, T. P. The use of *Xenopus* oocytes to probe synaptic communication. *Trends Neurosci.* **11**:250–256 (1988).
2. Gunderson, C. B., R. Miledi, and I. Parker. Messenger RNA from human brain induces drug- and voltage-operated channels in *Xenopus* oocytes. *Nature (Lond.)* **308**:421–424 (1984).
3. White, M. M., K. M. Mayne, H. A. Lester, and N. Davidson. Mouse-*Torpedo* hybrid acetylcholine receptors: functional homology does not equal sequence homology. *Proc. Natl. Acad. Sci. USA* **82**:4852–4856 (1985).
4. Mishina, M., T. Tobimatsu, K. Imoto, K. Tanaka, Y. Fujita, K. Fukuda, M. Kurasaki, H. Takahashi, Y. Morimoto, T. Hirose, S. Inayama, T. Takahashi, M. Kuno, and S. Numa. Location of functional regions of acetylcholine receptor α subunit by site-directed mutagenesis. *Nature (Lond.)* **313**:364–369 (1985).
5. Methfessel, C., V. Witzmann, T. Takahashi, M. Mishina, S. Numa, and B. Sakmann. Patch clamp measurements on *Xenopus laevis* oocytes: currents through endogenous channels and implanted acetylcholine receptor and sodium channels. *Pfluegers Arch.* **407**:577–588 (1986).
6. Buller, A. L., and M. M. White. Control of *Torpedo* acetylcholine receptor biosynthesis in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **85**:8717–8721 (1988).
7. White, M. M., and T. Resine. Expression of functional pituitary somatostatin receptors in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA*, **87**:133–136 (1990).
8. Miledi, R. A calcium-dependent transient outward current in *Xenopus laevis* oocytes. *Proc. R. Soc. Lond. B Biol. Sci.* **215**:491–497 (1982).
9. Dascal, N., T. P. Snutch, H. Lubbert, N. Davidson, and H. A. Lester. Expression and modulation of voltage-gated calcium channels after RNA injection in *Xenopus* oocytes. *Science (Wash. D. C.)* **231**:1147–1150 (1986).
10. Barish, M. E. A transient calcium dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol. (Lond.)* **342**:309–325 (1983).
11. Miledi, R., and I. Parker. Chloride current induced by injection of calcium in *Xenopus* oocytes. *J. Physiol. (Lond.)* **357**:173–183 (1984).
12. Manthey, A. A. The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. *J. Gen. Physiol.* **49**:963–976 (1966).
13. White, M. M. Forskolin alters acteylcholine receptor gating by a mechanism independent of adenylate cyclase activation. *Mol. Pharmacol.* **34**:427–430 (1988).
14. Kelso, S. R., and J. P. Leonard. "Desensitization" of NMDA receptors may involve chloride in *Xenopus* oocytes. *Soc. Neurosci. Abstr.* 218.15 (1989).
15. Hille, B. *Ionic Channels of Excitable Membranes*. Sinauer Associates, Sunderland, MA. 1984.
16. Knauf, P. A., and A. Rothstein. Chemical modification of membranes. I. Effects of sulfhydryl and amino reagents on anion and cation permeation of human red blood cells. *J. Gen. Physiol.* **58**:190–210 (1971).
17. Miller, C., and M. M. White. A voltage-gated Cl^- conductance channel from *Torpedo* electroplax membrane. *Ann. N. Y. Acad. Sci.* **341**:534–551 (1980).
18. Inoue, I. Voltage-dependent Cl^- conductance of the squid giant axon membrane and its blockade by some disulfonic stilbene derivatives. *J. Gen. Physiol.* **85**:519–537 (1985).
19. Inoue, I. Modification of K^+ conductance of the squid giant axon membrane by SITS. *J. Gen. Physiol.* **88**:507–520 (1986).
20. Palade, P. T., and R. L. Barchi. On the inhibition of muscle membrane chloride conductance by aromatic carboxylic acids. *J. Gen. Physiol.* **69**:879–896 (1977).
21. Cousin, J. L., and R. Motais. Inhibition of anion permeability by amphiphilic compounds in human red cell: evidence for an interaction of niflumic acid with the Band 3 protein. *J. Membr. Biol.* **46**:125–153 (1979).
22. Knauf, P. A., and N. A. Mann. Use of niflumic acid to determine the nature of the asymmetry of the human erythrocyte anion exchange system. *J. Gen. Physiol.* **83**:703–725 (1984).
23. Brule, G., G. Haudecoeur, H. Jdaiaa, and P. Guilbault. Inhibition par une substance amphiphile, l'acide niflumique, de la rectification dans le sens entrent de la fibre musculaire de Crustace. *Arch. Int. Physiol. Biochim.* **91**:269–277 (1983).
24. Dumont, J. N. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136**:153–180 (1972).
25. Auffray, C., and F. Rougeon. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem.* **107**:303–324 (1980).
26. Boton, R., N. Dascal, B. Gillo, and Y. Lass. Two calcium-activated chloride conductances in *Xenopus laevis* oocytes permeabilized with the ionophore A23187. *J. Physiol. (Lond).* **408**:511–534 (1989).
27. Edwards, C. The selectivity of ion channels in nerve and muscle. *Neuroscience* **7**:1335–1366 (1982).
28. Welsh, M. J. Anthracene-9-carboxylic acid inhibits an apical membrane chloride conductance in canine tracheal epithelium. *J. Membr. Biol.* **78**:61–71 (1984).
29. Evoniuk, G., and P. Skolnick. Picrate and niflumate block anion modulation of radioligand binding to the γ-aminobutyric acid/benzodiazepine receptor complex. *Mol. Pharmacol.* **34**:837–842 (1988).

Send reprint requests to: Dr. Michael M. White, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.