Neuroleptics Antagonize a Calcium-activated Potassium Channel in Airway Smooth Muscle

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ABSTRACT  We examined the effect of neuroleptics on Ca-activated K channels from dog airway smooth muscle cells. Because these agents inhibit a variety of other Ca-mediated processes, it seemed possible that they might also inhibit Ca-activated K channels. In excised, inside-out patches, several neuroleptics potently and reversibly inhibited the K channel from the internal but not the external surface of the patch. Measurements of the effect on open probability and open- and closed-state durations support a simple kinetic model in which neuroleptics bind to and block the open channel. Inhibition by neuroleptics was moderately voltage dependent, with blockers less potent at hyperpolarizing voltages. The relationship between voltage and the dissociation constant for the blocker suggests that the binding site is one-third of the way across the channel's electrical field. Equilibrium dissociation constants for the drug-channel complex were: haloperidol, 1.0 ± 0.1 μM; trifluoperazine, 1.4 ± 0.1 μM; thioridazine, 2.4 ± 0.1 μM; and chlorpromazine, 2.0 μM. This rank-order potency is different from their potency as calmodulin inhibitors, which suggests that neuroleptics bind to the channel rather than a calmodulin-channel complex.

INTRODUCTION

Neuroleptics are a class of drug used in the treatment of psychiatric diseases, particularly schizophrenia, and are thought to have their therapeutic effect by antagonizing dopamine receptors (Creese et al., 1975). Neuroleptics also inhibit calmodulin, the ubiquitous Ca-activated protein (Levin and Weiss, 1979). Because neuroleptics inhibit calmodulin, we thought it possible that they might also antagonize other Ca-activated proteins. Therefore, we examined their effect on another Ca-activated protein, the Ca-activated K channel in canine tracheal smooth muscle.

The Ca-activated K channel in airway smooth muscle (McCann and Welsh, 1986) has properties that are very similar to other large-conductance, Ca-activated K channels (Marty, 1981; Pallotta et al., 1981; Adams et al., 1982; Barrett et al., 1982; Latorre et al., 1982; Magleby and Pallotta, 1983; Maruyama et al., 1983; Yellen, 1984; Benham et al., 1985; Latorre and Miller, 1983). It
has a single channel conductance of 266 pS (in symmetrical 135 mM K solutions) and is highly selective for K over Na, Ca, and anions. Both depolarization and an increase in the Ca concentration bathing the cytosolic surface increase the probability of finding the channel open. The channel is blocked by tetraethylammonium, Ba, and Cs.

MATERIALS AND METHODS

Cell Culture

Isolated canine airway smooth muscle cells were obtained by enzymatic dispersion as previously described (McCann and Welsh, 1986). Cells were plated on fibronectin-coated 35-mm petri dishes at a density of 5 × 10⁴ cells/cm². The incubation media consisted of medium 199 Earle's base supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). The cells were incubated at 37°C and 6% CO₂ for 2–80 h before use. 20 primary cultures were prepared during the course of these experiments.

Single Channel Recording

Our technique for production of pipettes and seal formation is similar to that described by Hamill and co-workers (1981). The pipette resistance (when filled with 140 mM KCl) was 4–10 MΩ, and the average seal resistance was 10.3 ± 1.1 GΩ (mean ± SEM, n = 15). During seal formation, the cells were bathed in a HEPES-buffered NaCl Ringer's solution. After seal formation, patches were excised inside-out into this NaCl Ringer's. Discrete single channel events became apparent after drawing the patch pipettes through the air-water interface several times.

A model 8900 Dagan patch clamp (Dagan Corp., Minneapolis, MN) was used for voltage-clamping and current amplification. Currents were low-pass-filtered at 1 kHz through an eight-pole Bessel filter (Frequency Devices, Inc., Haverhill, MA). Currents were digitized at 2 kHz and stored on a PDP 11/23+ laboratory computer (Indec, Sunnyvale, CA).

Voltages are referenced to the outside of the plasma membranes. Outward current (cations flowing from the bathing solution into the pipette) is considered positive and is represented as an upward deflection in the figures.

Analysis

In order to simplify the analysis, patches of membrane containing more than two channels were discarded; this included >90% of all patches. The number of channels in a patch was determined by holding the patch at a voltage where the open-state probability was ~0.5. Under these conditions, it is easy to determine whether one, two, or more channels are present. The digitized currents were analyzed by two independent methods.

If only one channel was present in the patch, a half-amplitude crossing analysis was done to obtain probability density histograms for both the open and closed states. The probability density histograms were fitted to single exponentials by the method of maximum likelihood (Colquhoun and Hawkes, 1983). Only openings and closings longer than 2 ms were used to make the maximum-likelihood estimates. The estimate was then corrected for events (in the same distribution) shorter than 2 ms (Colquhoun and Sigworth, 1983); however, interval omission errors were not corrected for, and no attempt was made to idealize the data before analysis. It should be noted that more than one open and closed state have been described for large-conductance, Ca-activated K channels from other tissues (Magleby and Pallotta, 1983) and probably also exist in channels from airway smooth muscle. However, as will be discussed subsequently, with the conditions (see below)
and the filtering frequency used here, the open state could be described as a single exponential.

When patches of membrane contained either one or two channels, the probability of the channel being found in the open state was obtained by constructing amplitude histograms of the digitized currents. The area under each peak in the histogram was then integrated to obtain the probability of zero, one, and two channels being open. If only one channel was present, the probability of the channel being open could be obtained directly. If two channels were present in the patch, the open-state probability was obtained assuming independence of the two channels (Colquhoun and Hawkes, 1983).

Data from 16 patches have been used in this report. In all experiments, the voltage across patches of membrane was stepped from 0 to +40 mV (unless stated otherwise) for 4.09 s. Channel open probability and open- and closed-state durations are stable by the time the capacitative transient is completed. Only the last 3.9 s of sweeps was used for analysis. Occasionally, a channel would enter a long deactivated state (>100 ms); sweeps containing long closures were not included in the analysis. It is possible that this resulted from Ca blocking the channel (Magleby and Pallotta, 1983). Patches of membrane that contained a significant number (>10% of the sweeps) of long closed states were discarded; 15% of all patches fell into this category.

Solutions
The NaCl Ringer’s solution used to bathe the cells during seal formation contained 140 mM Na, 1.2 mM Ca, 1.2 mM Mg, and 145 mM Cl. After excising the patch of membrane, inside out, the bathing solution was exchanged with a solution containing 155 mM K, 1 mM Ca, and 137 mM Cl. We chose this solution containing a high Ca concentration because preliminary studies showed it to provide the most reproducible and stable channel kinetics and the greatest probability of finding the channel open. The solution used to fill the pipette contained 135 mM K, 5 mM Mg, and 145 mM Cl. Solutions also contained HEPES (10 mM) buffered to a pH of 7.2 with KOH (5 mM) in room air.

Drugs were prepared in aqueous stock solutions daily and stored in the dark. Solution exchanges were accomplished by exchanging the 2 ml of solution in the experimental chamber with a 30-ml vol at a flow rate of 1 ml/s. All experiments were done at room temperature (21–23°C).

Reagents
We used the following compounds: fetal calf serum (Cancer Center Tissue Culture Lab [CCTCL], University of Iowa), HEPES (Sigma Chemical Co., St. Louis, MO), medium 199, Earle’s Base (CCTCL), penicillin and streptomycin (CCTCL), haloperidol (Sigma Chemical Co.), and thioridazine hydrochloride (Aldrich Chemical Co., Milwaukee, WI). Trifluoperazine hydrochloride, chlorpromazine hydrochloride, and methochlorpromazine were generous gifts from Smith, Kline and French Labs (Philadelphia, PA).

RESULTS
Fig. 1 shows representative examples of the effect of 1-μM concentrations of haloperidol, trifluoperazine, thioridazine, and chlorpromazine applied to the cytoplasmic surface of an excised patch of membrane. This figure also shows the effect of 2 μM trifluoperazine present in the pipette solution (the external surface of the patch). The first tracing demonstrates that under these experimental conditions, the channel was open nearly all the time, and the openings were separated by brief flickers into a nonconducting or closed state. The addition of each of the neuroleptics to the cytoplasmic side of the patch decreased the probability of finding the channel in the open state. It is apparent that internal
application of drugs also decreased the mean open duration. A comparison of
the first three tracings suggests that haloperidol and trifluoperazine also increase
the mean closed duration.

Outside-out patches of membrane could not be obtained to examine the effect
of these agents on the external surface of the channel. Therefore, neuroleptics

were added to the pipette solution. The fourth tracing (in Fig. 1) shows a patch
in which 2 μM trifluoperazine was added to the external (pipette) solution; there
was no apparent effect in three experiments. Thus, if these drugs do have an
effect when applied to the external surface of the channel, they are much less
potent from the outside.

FIGURE 1. Effect of neuroleptics on the Ca-activated K channel. Representative
tracings were obtained under control conditions and when the internal (bath)
solution contained the indicated concentrations of haloperidol, trifluoperazine,
thioridazine, and chlorpromazine. The fourth tracing was obtained when trifluo-
perazine was present in the external (pipette) solution. Tracings were recorded at
+40 mV and are excerpts from 4-s voltage sweeps. Upward deflections represent
channel openings. The top three tracings were obtained from the same patch and
the bottom two tracings were obtained from a different patch. The dashed line
indicates the zero-current level.
A half-amplitude crossing analysis was performed on single channel current records like those shown in Fig. 1 in order to construct open- and closed-lifetime distributions. Fig. 2 shows three representative probability density histograms from the same patch that generated the first three tracings in Fig. 1. The open-state-duration histograms could always be fitted by single exponentials under

**FIGURE 2.** Effect of increasing haloperidol concentrations on the probability density histogram of the open-state durations. Histograms were obtained from the same patch that generated the first three tracings in Fig. 1. Haloperidol concentrations are as indicated. Note the change in the time base for the third histogram: the histograms were constructed from 1,532 (A), 1,770 (B), and 3,952 (C) observations.
these experimental conditions. (Note that events shorter than 2 ms were discarded.) The solid line is a maximum-likelihood estimate of the single-exponential distribution that best describes the data. The addition of haloperidol decreased the open-state-duration time constant in a dose-dependent manner. Under control conditions, the open-state-duration time constant was 41.6 ms; 0.1 μM haloperidol decreased the time constant to 21.7 ms and 1 μM haloperidol decreased the time constant to 5.9 ms.

**Figure 3.** Effect of increasing haloperidol concentrations on the probability density histogram of the closed-state durations. Histograms were obtained from the same patch used for Fig. 2.
Fig. 3 shows representative examples of the distribution of closed-state durations taken from the same patch used in Fig. 2. Under control conditions, the distribution of closed-state durations could not be fitted with a single exponential. This observation is in agreement with the work of others suggesting the presence of multiple closed states (Magleby and Pallotta, 1983). We made no attempt to fit the closed-state-duration distribution with the sum of several exponentials. Adding 0.1 μM haloperidol increased the mean closed duration from 1.6 to 4.4 ms, but the distribution still could not be fitted with a single exponential. However, with 1 μM haloperidol, the closed-state probability density distribution could be described by a single exponential with a time constant of 10 ms.

Table I summarizes the time constants for the open- and closed-state distributions under control conditions and in the presence of various concentrations of haloperidol, trifluoperazine, and thioridazine. Under all conditions, the open-state distribution was well fitted by a single exponential. Increasing the concentration of these agents bathing the internal side of the channel decreased the open-state time constant.

In contrast, the closed-state-duration distribution was not single exponential under control conditions or in the presence of low concentrations of neuroleptics. Low concentrations of these agents increased the mean closed time and, at higher concentrations, the closed time was fitted by a single exponential. No further increase in the time constant describing the closed state was observed with haloperidol concentrations >1 μM, trifluoperazine concentrations >2 μM, or thioridazine concentrations >5 μM (data not shown). Most of the closed-state durations in the presence of chlorpromazine were too brief to reach full amplitude in the frequency bandwidth used; thus, using a half-amplitude crossing analysis would lead to large inaccuracies because of interval omission errors.

| Condition | \( T_{open} \) | \( T_{closed} \) | \( P \) | \( N \) |
|-----------|-----------------|-----------------|-------|------|
| Control   | 60.3±5.5        | —               | 0.97±0.8 | 8    |
| [Haloperidol] (μM) |                  |                 |       |      |
| 0.1       | 26.3±2.9        | —               | 0.87±0.01 | 5    |
| 0.2       | 22.2±2.6        | —               | 0.79±0.01 | 5    |
| 0.5       | 13.8±2.3        | —               | 0.64±0.02 | 5    |
| 1         | 7.7±1.0         | 9.4±0.5         | 0.48±0.03 | 5    |
| [Trifluoperazine] (μM) |                  |                 |       |      |
| 0.2       | 22.0±3.3        | —               | 0.88±0.03 | 5    |
| 0.5       | 12.5±1.9        | —               | 0.68±0.03 | 5    |
| 1         | 8.1±1.6         | —               | 0.58±0.05 | 5    |
| 2         | 3.3±0.4         | 3.9±0.4         | 0.41±0.02 | 5    |
| [Thioridazine] (μM) |                  |                 |       |      |
| 1         | 5.7±0.8         | —               | 0.72±0   | 2    |
| 2         | 2.6±0.4         | —               | 0.58±0.03 | 2    |
| 5         | 1.1±0.2         | 2.7±0.1         | 0.32±0.01 | 2    |
| [Chlorpromazine] (μM) |                  |                 |       |      |
| 1         | —               | —               | 0.83±0.02 | 7    |
| 2         | —               | —               | 0.76±0.02 | 7    |
| 5         | —               | —               | 0.63±0.04 | 5    |
Therefore, we did not obtain lifetime distribution histograms for single channel currents in the presence of chlorpromazine.

Table I also shows the effect of the neuroleptics on the probability of finding the channel open \((P)\). Increasing the concentration of each of the agents decreased the probability that the channel was open. The determination of probability using histograms of the currents is less dependent on the frequency response of the recording system than the crossing analysis. Therefore, we were able to determine the probability of the channel being open in the presence of several concentrations of chlorpromazine as well as the other agents. The effect of these drugs was also largely reversible. In three experiments, \(P\) under control conditions was \(0.96 \pm 0.01\), decreased to \(0.41 \pm 0.04\) in the presence of \(1 \mu M\) haloperidol, and then increased to \(0.92 \pm 0.01\) after haloperidol was washed out.

We also examined the voltage dependence of channel inhibition by neuroleptics. Table II shows the probability of finding the channel open at six voltages under control conditions and in the presence of \(1 \mu M\) haloperidol. Under these control conditions (1 mM Ca), the probability of finding the channel open was very high and nearly independent of voltage. The charged neuroleptic haloperidol (valence +1 at pH 7.2) decreased the probability of the channel being open in a voltage-dependent manner; haloperidol was less potent at hyperpolarizing voltages. We found that inhibition by chlorpromazine and methochlorpromazine had a similar voltage dependence (data not shown).

**TABLE II**

| Voltage | \(P\) \(\pm\)0.01 | \(P_{\text{haloperidol}}\) \(\pm\)0.05 |
|---------|------------------|---------------------|
| 60      | 0.97 \(\pm\)0.01 | 0.45 \(\pm\)0.03   |
| 40      | 0.97 \(\pm\)0.01 | 0.49 \(\pm\)0.02   |
| 20      | 0.96 \(\pm\)0.01 | 0.53 \(\pm\)0.02   |
| −20     | 0.94 \(\pm\)0.01 | 0.62 \(\pm\)0.04   |
| −40     | 0.90 \(\pm\)0.02 | 0.65 \(\pm\)0.04   |
| −60     | 0.83 \(\pm\)0.06 | 0.64 \(\pm\)0.05   |

D I S C U S S I O N

We found that four neuroleptics antagonized the Ca-activated K channel activity in canine airway smooth muscle. This effect was concentration dependent, reversible, and specific to the internal surface of the membrane. Each of the drugs decreased the probability that the channel was open and decreased the time constant of the distribution of open states. We also observed that, under control conditions, the distribution of the closed durations could not be fitted by a single exponential, but upon increasing the drug concentration, the distribution became single exponential.

The interaction of a drug with a channel protein can produce a nonconducting state in several ways. One mechanism is block of a channel, which is usually taken to mean obstruction of a channel. As reviewed by Yellen (1984), blocking drugs can decrease current in ways that appear different based on the length of time
that the blocker obstructs the channel. For example, agents such as Ba and nonyltriethylammonium produce a slow block that appears similar to the normal closing of the channel. Other agents, such as Cs, appear to decrease the open channel current, presumably by producing such rapid intermittent blocking that it cannot be readily resolved. A third mechanism of block produces a current tracing in which the channel appears to "flicker," because the duration of the block is long enough to detect, but too short to completely resolve. Another mechanism by which a drug could decrease channel current is by altering the gating properties of a channel by changing the rate constants for transitions between different conducting states. We cannot discriminate between a slow block or some mechanism by which channel gating is altered. Therefore, for the purposes of the following discussion, we have chosen to call the nonconducting state a blocked state.

We will now consider our findings in terms of a simple kinetic model that is consistent with the observations:

\[
\text{Closed}_N \rightleftharpoons \text{Open} + \text{Drug} \\
\frac{k_1}{k_{-1}} \text{ Blocked}
\]

The model consists of a channel that has a single open state and several closed states. \(k_1\) is the on rate for a given drug and \(k_{-1}\) is the off rate, or the rate at which the drug-channel complex dissociates. One open state is proposed for the channel because under all conditions the distribution of the open durations was single exponential (Fig. 2 and Table I). This is also consistent with studies of large Ca-activated K channels from arterial and intestinal smooth muscle studied with similar bandwidth and conditions (Benham et al., 1985). More than one closed state is proposed because, under control conditions, the distribution of the closed state was not well described by a single exponential. We did not attempt to determine the number of closed states for the channel, but as many as four distinguishable nonconductive states have been proposed for a similar Ca-activated K channel found in skeletal muscle (Magleby and Pallotta, 1983). Each of the four neuroleptics described in the study is able to bind to the open channel, creating a unique nonconductive state labeled "blocked" in Eq. 1.

The model proposed in Eq. 1 predicts the following relationship:

\[
1/T_{\text{open}} = k_1 [\text{drug}] + k_{\text{tot}},
\]

where \(T_{\text{open}}\) is the time constant describing the distribution of the open states, \(k_1\) is the drug on rate, and \(k_{\text{tot}}\) is the sum of all rate constants leading to closed but unblocked states. Thus, a plot of \(1/T_{\text{open}}\) vs. drug concentration should yield a line with a slope of \(k_1\) (Neher and Steinbach, 1978). The data from Table I were plotted in Fig. 4 to demonstrate that such a plot is linear. The linear regression analysis of the data shown in Fig. 4 yields drug on rates of \(0.11 \pm 0.01\) ms \(\mu\)M\(^{-1}\) for haloperidol, \(0.15 \pm 0.02\) ms \(\mu\)M\(^{-1}\) for trifluoperazine, and \(0.19 \pm 0.03\) ms \(\mu\)M\(^{-1}\) for thioridazine.

Eq. 1 also predicts that at sufficiently high drug concentrations, nearly every transition of the channel into a nonconducting state should be due to channel blockade. Thus, when \(k_1 [\text{drug}] \gg k_{\text{tot}}\), the distribution of the closed states should
be fitted by a single exponential with a time constant equal to \(1/k_1\). Fig. 3 and Table I show that this is the case, which suggests that at high drug concentrations, \(k_1[\text{drug}] \gg k_{\text{off}}\). The off rate determined by this method was \(0.108 \pm 0.005 \text{ ms}^{-1}\) for haloperidol, \(0.27 \pm 0.03 \text{ ms}^{-1}\) for trifluoperazine, and \(0.38 \pm 0.01 \text{ ms}^{-1}\) for thioridazine.

![Graph showing relation between increasing concentrations of neuroleptics and the channel closing rate constant](image)

**Figure 4.** Relation between increasing concentrations of neuroleptics and the channel closing rate constant (the inverse of the time constant of the open-state duration). Values represent means ± SEM for: five experiments for haloperidol (A), five experiments for trifluoperazine (B), and two tissues for thioridazine (C). Lines were fitted by least-squares linear regression analysis. Slopes and intercepts, respectively, for the lines are: haloperidol, \(0.108 \text{ ms}^{-1} \mu\text{M}^{-1}\) and \(0.022 \pm 0.003 \text{ ms}^{-1}\); trifluoperazine, \(0.140 \text{ ms}^{-1} \mu\text{M}^{-1}\) and \(0.010 \pm 0.012 \text{ ms}^{-1}\); thioridazine, \(0.180 \text{ ms}^{-1} \mu\text{M}^{-1}\) and \(0.012 \pm 0.011 \text{ ms}^{-1}\).

The equilibrium dissociation constant for the drug-channel complex, \(K_d\), can be estimated from the ratio \(k_1/k_{\text{off}}\). The \(K_d\) values for haloperidol, trifluoperazine, and thioridazine estimated by this method were \(1.0 \pm 0.1, 1.9 \pm 0.2,\) and \(2.0 \pm 0.3 \mu\text{M},\) respectively.
Values for the $K_d$ can also be estimated by an independent method using only the probability of the channel being open at various drug concentrations. The model shown in Eq. 1 predicts that the following relationship should be satisfied (Benham et al., 1985):

$$K_d = \frac{[\text{drug}](P \cdot P_d)}{(P - P_d)},$$

(3)

where $P$ is the probability of the channel being open in the absence of drug, $P_d$ is the probability of the channel being open in the presence of drug, and $[\text{drug}]$ is the concentration of the neuroleptic. A plot of drug concentration vs. $(P - P_d)/(P \cdot P_d)$ should be linear with a slope equal to $1/K_d$. Fig. 5 demonstrates that these plots are linear, with zero intercepts for each of the neuroleptics. The $K_d$ values estimated by this method were $1.0 \pm 0.1 \, \mu M$ for haloperidol, $1.4 \pm 0.2 \, \mu M$ for trifluoperazine, $2.4 \pm 0.1 \, \mu M$ for thioridazine, and $8.7 \pm 2.1 \, \mu M$ for chlorpromazine. These estimates of $K_d$ are in excellent agreement with those calculated from the drug on and off rates ($1.0 \, \mu M$ for haloperidol, $1.9 \, \mu M$ for trifluoperazine, and $2.0 \, \mu M$ for thioridazine). The close agreement between the values of $K_d$ further supports the model shown in Eq. 1.

The analysis presented above supports the simple model of Eq. 1 for the interaction of neuroleptics with the Ca-activated K channel. However, other models are possible, particularly if one assumes that the drug affects more than one rate constant.

Fig. 6 shows the effect of voltage on $K_d$ for haloperidol. The data come from Table II ($n = 4$), with $1 \, \mu M$ haloperidol added; in addition, three experiments are included in which $0.5 \, \mu M$ haloperidol was added. $K_d$ was calculated using Eq. 3. The $K_d$ for block was voltage dependent, with haloperidol becoming less potent at hyperpolarizing voltages. This finding suggests that the binding site
for the drug is located within the channel's electrical field. The relationship describing the voltage dependence of such a block is (Woodhull, 1973):

\[ K_d(V) = K_d(0 \text{ mV}) \exp(-dzFV)/(RT) \]

where \( K_d(0 \text{ mV}) \) is the dissociation constant at 0 mV extrapolated from Fig. 6, \( d \) is the distance across the channel's electrical field at which drug binding occurs, \( z \) is the valence of the drug, and \( F, V, R, \) and \( T \) have their usual meanings. Using data from Fig. 6, we obtained a value of \( d = 0.31 \). This suggests that the blocking site for haloperidol lies about one-third of the way across the channel's electrical field.

Neuroleptics specifically inhibit several other Ca-mediated cell processes. For example, neuroleptics of the diphenylpiperidine class are potent Ca channel antagonists (Gould et al., 1983), and the calmodulin antagonist R24571 inhibits the Ca/Mg ATPase and Ca-dependent protein kinase (Lamers and Stinis, 1983). High concentrations of neuroleptics, including phenothiazines and haloperidol, also nonspecifically inhibit lipid-bound proteins (Weiss et al., 1982). However, it seems unlikely that the inhibition of the K channel resulted from a nonspecific effect. This conclusion is supported by three observations: (a) the concentrations used in this study are lower than those associated with nonspecific membrane effects (Weiss et al., 1982); (b) inhibition by these drugs was specific to the cytoplasmic side of the membrane; and (c) the kinetic data are consistent with a simple bimolecular reaction between the channel and the drug.

The neuroleptics drugs represent a new class of antagonists for the large Ca-activated K channel. The four drugs described in this study bind to the open channel with dissociation constants in the micromolar range. The only other high-affinity antagonist of the large Ca-activated K channel is a minor component of venom from the Israeli scorpion, *Leiurus quinquestriatus*, called charybdotoxin (Miller et al., 1985).

It has been suggested that calmodulin may be associated with the K channel, thereby giving the channel its Ca sensitivity (Lackington and Orrego, 1981). The...
rank-order potency for three of the drugs as calmodulin antagonists is trifluoperazine > chlorpromazine > haloperidol (Levin and Weiss, 1979). In contrast, the rank-order potency as K channel antagonists is haloperidol > trifluoperazine > chlorpromazine. A comparison of these rank-order potencies does not support the notion that calmodulin is associated with this channel. Rather, it suggests that these drugs may interact directly with the channel.

It will be interesting to see whether neuroleptics inhibit Ca-activated K channels in other preparations. We have observed that micromolar concentrations of haloperidol have no effect on a voltage-insensitive, inwardly rectifying, Ca-activated K channel from the basolateral membrane of dog and human tracheal epithelia (Welsh and McCann, 1985, and unpublished observation). It will also be interesting to see whether the dissociation constants for a wide variety of neuroleptics correlate with clinical potency or with the occurrence of extrapyramidal side effects associated with the widespread clinical use of these drugs.

We thank Phil Karp and Timothy R. Ruppert for excellent technical assistance, and Vanessa Krumbholz for secretarial assistance.

This work was supported by the National Heart, Lung, and Blood Institute (HL-35804). J.D.M. is an American Heart Association Student Fellow. M.J.W. is an Established Investigator of the American Heart Association.

Original version received 20 June 1986 and accepted version received 28 August 1986.

REFERENCES

Adams, P. R., A. Constanti, D. A. Brown, and R. B. Clark. 1982. Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurons. Nature. 296:746–749.

Barrett, J. N., K. L. Magleby, and B. S. Pallotta. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. Journal of Physiology. 331:211–230.

Benham, C. D., T. B. Bolton, R. J. Lang, and T. Takewaki. 1985. The mechanism of action of Ba^{2+} and TEA on single Ca^{2+}-activated K^+-channels in arterial and intestinal smooth muscle cell membranes. Pflügers Archiv. 403:120–127.

Colquhoun, D., and A. G. Hawkes. 1983. The principles of the stochastic interpretation of ion-channel mechanisms. In Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 135–175.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. In Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 191–263.

Creese, I., D. R. Burt, and H. S. Solomon. 1975. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science. 192:481–482.

Gould, R. J., K. M. M. Murphy, I. J. Reynolds, and H. S. Solomon. 1983. Antischizophrenic drugs of the diphenylbutylpiperidine type act as calcium channel antagonists. Proceedings of the National Academy of Sciences. 80:5122–5125.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Archiv. 391:85–100.

Lackington, I., and F. Orrego. 1981. Inhibition of calcium-activated potassium conductance of human erythrocytes by calmodulin inhibitory drugs. FEBS Letters. 133:103–106.
Lamers, J. M. J., and J. T. Stinis. 1983. Inhibition of Ca\textsuperscript{2+}-dependent protein kinase and Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-ATPase. *Cell Calcium.* 4:281–294.

Latorre, R., and C. Miller. 1983. Conduction and sensitivity in potassium channels. *Journal of Membrane Biology.* 71:11–30.

Latorre, R., C. Vergara, and C. Hidalgo. 1982. Reconstitution in planar lipid bilayers of a Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel from transverse tubule membranes isolated from rabbit skeletal muscle. *Proceedings of the National Academy of Sciences.* 79:805–809.

Levin R. M., and B. Weiss. 1979. Selective binding of antipsychotics and other psychoactive agents to the calcium-dependent activator of cyclic nucleotide phosphodiesterase. *Journal of Pharmacology and Experimental Therapeutics.* 208:454–459.

Magleby, K. L., and B. S. Pallotta. 1983. Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *Journal of Physiology.* 344:585–604.

Marty, A. 1981. Ca-dependent K channels with large unitary conductance in chromaffin cell membranes. *Nature.* 291:497–500.

Maruyama, Y., O. H. Petersen, P. Flanagan, and G. T. Pearson. 1983. Quantification of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels under hormonal control in pig pancreas acinar cells. *Nature.* 305:228–232.

McCann, J. D., and M. J. Welsh. 1986. Calcium activated potassium channels in canine airway smooth muscle. *Journal of Physiology.* 372:115–127.

Miller, C., E. Moczydlowski, R. Latorre, and M. Phillips. 1985. Charybdotoxin, a protein inhibitor of single Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels from mammalian skeletal muscle. *Nature.* 313:316–318.

Neher, E., and J. H. Steinbach. 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *Journal of Physiology.* 277:155–176.

Pallotta, B. S., K. L. Magleby, and J. N. Barrett. 1981. Single channel recordings of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents in rat muscle cell culture. *Nature.* 293:471–474.

Weiss, B., W. C. Prozialeck, and T. L. Wallace. 1982. Interaction of drugs with calmodulin: biochemical, pharmacologic and clinical implications. *Biochemical Pharmacology.* 31:2217–2226.

Welsh, M. J., and J. D. McCann. 1985. Intracellular calcium regulates basolateral potassium channels in a chloride secreting epithelium. *Proceedings of the National Academy of Sciences.* 24:8823–8826.

Woodhull, A. M. 1975. Ionic blockade of sodium channels in nerve. *Journal of General Physiology.* 61:687–708.

Yellen, G. 1984. Ionic permeation and blockade in Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels of bovine chromaffin cells. *Journal of General Physiology.* 84:157–186.