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Characterization of Inhibition by Risperidone of the Inwardly Rectifying K^+ Current in Pituitary GH_3 Cells

Sheng-Nan Wu, M.D., Ph.D., Chung-Ren Jan, Ph.D., Hui-Fang Li, B.S., and Hung-Ting Chiang, M.D.

The effects of risperidone on ionic currents in rat pituitary GH_3 cells were investigated with the aid of the patch-clamp technique. Hyperpolarization-activated K^+ currents in GH_3 cells bathed in high-K^+ Ca^{2+}-free solution were studied to determine the effect of risperidone and other related compounds on the inwardly rectifying K^+ current ($I_{K(IR)}$). Risperidone (0.1–10 μM) suppressed the amplitude of $I_{K(IR)}$ in a concentration-dependent manner. The IC_{50} value for the risperidone-induced inhibition of $I_{K(IR)}$ was 1 μM. Risperidone (3 μM) was found to slow the rate of activation. An increase in current deactivation by the presence of risperidone was also observed. Haloperidol (10 μM) and thioridazine (10 μM) inhibited the amplitude of $I_{K(IR)}$ effectively, and clozapine slightly suppressed it; however, metoclopramide (10 μM) had no effect on it. Risperidone (10 μM) had no effect on voltage-dependent K^+ and L-type Ca^{2+} currents. However, in the inside-out configuration, risperidone (10 μM) did not alter the single-channel conductance, but reduced the activity of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels. Under the current-clamp mode, risperidone (3 μM) depolarized the membrane potential and increased the firing rate. With the aid of the spectral analysis, cells that exhibited an irregular firing pattern were also converted to those displaying a regular firing pattern after addition of risperidone (3 μM). The present study provides evidence that risperidone, in addition to the blockade of dopamine receptors, can produce a depressant effect on $I_{K(IR)}$ and BK_{Ca} channels, and implies that the blockade of these ionic currents by risperidone may affect membrane excitability and prolactin secretion in GH_3 cells. ***[Neuropsychopharmacology 23:676–689, 2000]***

KEY WORDS: *Risperidone; Inwardly rectifying K^+ current; GH_3 cells*

Risperidone, a benzisoxazole compound that has been approved for use in the Unites States in 1994, is known to be effective in terminating acute psychotic episodes and preventing recurrence of psychotic episodes in schizophrenics (Chouinard et al. 1993; Breier et al. 1999). The most significant and consistent neuroendocrine effect of neuroleptic drugs is to stimulate prolactin secretion and cause galactorrhea, although these adverse effects vary greatly in potency and chemical structure (Bowden et al. 1992; Popli et al. 1998; Breier et al. 1999; Kleinberg et al. 1999; Petty 1999). This important site of action has been thought to be primarily due to the blockade of dopamine D_2 receptors (Popli et al. 1998; Kapur et al. 1999; Kim et al. 1999; Petty, 1999; Vanhauwe et al. 1999). On the other hand, recent evidence suggests that these neuroleptics, including risperidone, may cause a significant prolongation of electrocardiographic QT interval (Drici et al. 1998).

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In general, the selective blockade of a rapidly activating component of the delayed rectifier K^+ current manifests as a prolongation of action potential duration in cardiac myocytes and its electrocardiographic surrogate, the QT interval (Hondeghem and Synders 1990). It is of importance to mention that the human *ether-à-go-go*-related gene (*HERG*) has also been found to be primarily responsible for this type of K^+ current (Sanguinetti et al. 1995) and that haloperidol can block *HERG* channel expressed in frog oocytes (Suessbrich et al. 1997). Taken together, the possibility has arisen that in addition to their effects on the bindings to the receptors, the neuroleptic agents might be the regulators of ionic channels.

Pituitary GH_3 lactotrophs, in addition to the presence of voltage-dependent K^+ and Ca^{2+} currents, have been found to exhibit the inwardly rectifying K^+ current ($I_{K(IR)}$) (Bauer et al. 1990; Wu et al. 1998) which, on the basis of biophysical and pharmacological properties, was identified as an *erg*-mediated K^+ current (Schäfer et al. 1999; Schwarz and Bauer 1999). This current was sensitive to caffeine, thyrotropin-releasing hormone, and class III antiarrhythmic agents (e.g., E-4031), and proposed to be an important determinant of the resting membrane potential (Bauer et al. 1990; Barros et al. 1994, 1996, 1997; Weinsberg et al. 1997; Bauer 1998; Schwarz and Bauer 1999). More importantly, the blockade of $I_{K(IR)}$ was noted to produce an increase in the firing rate of action potentials (Barros et al. 1996; Weinsberg et al. 1997; Schwarz and Bauer 1999) and to stimulate prolactin secretion in rat lactotrophs (Bauer et al. 1999).

Previous studies have reported that risperidone stimulated prolactin release in pituitary cells (Bowden et al. 1992), and that haloperidol and chlopromazine suppressed voltage-dependent K^+ current in rat pheochromocytoma cells (Nakazawa et al. 1995). A recent report also showed that risperidone effectively blocked the inwardly rectifying *erg* current in native rat lactotrophs (Schäfer et al. 1999). To date, however, there is little, if any, evidence to suggest that there is the underlying mechanism of actions of the neuroleptic drugs (e.g., risperidone) on ionic currents in pituitary lactotrophs.

Therefore, in the present study, the electrophysiological effects of risperidone and other related compounds in GH_3 cells were investigated. We sought to: 1) determine whether risperidone has any effect on the *erg*-like inwardly rectifying K^+ current ($I_{K(IR)}$) in GH_3 cells; 2) compare the potency of other related compounds in blocking the amplitude of $I_{K(IR)}$; 3) examine the effect of risperidone on other types of ionic currents, including voltage-dependent K^+ and L-type Ca^{2+} currents, and large-conductance Ca^{2+}-activated K^+ channels; and 4) ascertain whether risperidone can influence the membrane potential and the firing pattern of spontaneous action potentials in these cells. The present results clearly indicate that the underlying risperidone-induced inhibition of $I_{K(IR)}$ in GH_3 cells is not associated with its blockade of dopamine receptors and would significantly contribute to the change in membrane potential, thus affecting prolactin secretion.

MATERIALS AND METHODS

Cell Culture

The clonal strain GH_3 cell line, originally derived from a rat anterior pituitary adenoma, was obtained from Culture Collection and Research Center (CCRC-60015; Hsinchu, Taiwan, ROC (Wu et al. 1998). GH_3 cells were grown in monolayer culture in 50-ml plastic culture flasks in a humidified environment of 5% CO_2/95% air in 5 ml of Ham's F-12 nutrient media (Life Technologies, Grand Island, NY). The media were supplemented with 15% heat-inactivated horse serum (v/v), 2.5% fetal calf serum (v/v), and 2 mM L-glutamine (Life Technologies). Cells were subcultured once a week, and a new stock line was generated from frozen cells (frozen in 10% glycerol in medium plus serum) every three months. The experiments were performed after five or six days of subcultivation (60–80% confluence).

Electrophysiological Measurements

Immediately before each experiment, GH_3 cells were dissociated and an aliquot of the cell suspension was transferred to a recording chamber mounted on the stage of an inverted microscope (Diaphot 200; Nikon, Tokyo, Japan). The microscope was coupled to a video camera system with magnification up to 1500X to continuously monitor cell size during the experiments. Cells were bathed at room temperature (20–25°C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Patch pipettes were prepared from Kimax capillary tubes (Vineland, NJ) using a vertical two-step electrode puller (PP-83; Narishige, Tokyo, Japan), and the tips were fire-polished with a microforge (MF-83; Narishige). The resistance of the patch pipette was 3 to 5 MΩ when immersed in normal Tyrode's solution.

A programmable stimulator (SMP-311; Biologic, Claix, France) was used to digitally generate the voltage pulses. Ionic currents were recorded with glass pipettes in the whole-cell or inside-out configuration of the patch-clamp technique with an RK-400 patch amplifier (Biologic) (Hamill et al. 1981; Wu et al. 1999a). All potentials were corrected for liquid junction potential, a value that would develop at the tip of the pipette when the composition of pipette solution was different from that of bath. Tested drugs were applied through perfusion or added to the bath to obtain the final concentration indicated.

Data Recording and Analysis

The signals consisting of voltage and current tracings were monitored with a digital storage oscilloscope (model 1602; Gould, Valley View, OH) and recorded on-line using a digital audio tape recorder (model 1204; Biologic). After the experiments, the stored data were fed back and digitized at 5 to 10 kHz with a Digidata 1200 analog-to-digital device (Axon Instruments, Foster City, CA) interfaced to a Pentium III-grade computer and pCLAMP 8.0 software package (Axon Instruments). Ionic currents were analyzed off-line with the aid of the Clampfit subroutine of pCLAMP or the Origin 6.0 software (Microcal, Northampton, MA).

To calculate percentage inhibition of risperidone on $I_{K(IR)}$, cells were bathed in high-K^+, Ca^{2+}-free solution and each cell was hyperpolarized from −10 to −120 mV. The current amplitudes during the application of risperidone were compared with those measured after a subsequent application of E-4031 (10 μM). E-4031 is known to be a selective blocker of $I_{K(IR)}$ (Weinsberg et al. 1997). The concentration of risperidone required to inhibit 50% of current amplitude was fitted to a Hill equation:

$$y = E_{max}/\{1 + (IC_{50}{}^{n}/[D]^{n})\},$$

where [D] is the concentration of risperidone, IC_{50} and n are the half-maximal concentration of risperidone required to inhibit $I_{K(IR)}$ (i.e., E-4031-sensitive current) and the Hill coefficient (slope factor), respectively, and E_{max} is risperidone-induced maximal inhibition of $I_{K(IR)}$.

Single-channel currents of large-conductance Ca^{2+}-activated K^+ channels were analyzed with Fetchan and Pstat subroutines in the pCLAMP software (Axon Instruments). Multigaussian adjustments of the amplitude distributions between channels were used to determine unitary currents. The functional independence between channels was verified by comparing the observed stationary probabilities with the values calculated according to the binomial law. The number of active channels in the patch N was counted at the end of each experiment through perfusion of a solution with 100 μM Ca^{2+} and then used to normalize opening probability at each potential. The opening probabilities were evaluated using an iterative process to minimize the χ^2 calculated with a sufficiently large number of independent observations.

The alteration in membrane potentials of GH_3 cells was examined under the current-clamp conditions. The frequency of spontaneous action potentials was characterized by transforming the oscillating signals from their time domain to their representation in the frequency domain with the aid of power spectral analysis. Spectral analysis was done based on a discrete Fourier transform algorithm with the aid of Origin software (Microcal) (Wu et al. 1996). When spontaneous action potentials in GH_3 exhibited a regular discharge pattern, a concentrated peak shown in the power spectrogram would correspond to the mean firing rate.

All values are reported as means ± S.E.M. The paired or unpaired Student's t-test and one-way ANOVA with the least-significance-difference method for multiple comparison were used for the statistical evaluation of differences among the mean values. Differences between the values were considered significant at a value of $p < .05$ or $< .01$.

Drugs and Solutions

Risperidone (3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyridol[1,2-a]pyrimidin-4-one), haloperidol (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone), clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine), thioridazine, metoclopramide, dopamine, and serotonin were purchased from Research Biochemicals International (Natick, MA). Tetraethylammonium chloride and tetrodotoxin were purchased from Sigma Chemical (St. Louis, MO). E-4031 and penitrem A were obtained from Biomol (Plymouth Meeting, PA). All other chemicals were commercially available and of reagent grade.

The composition of normal Tyrode's solution was 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 5.5 mM glucose, and 5.5 mM HEPES-NaOH buffer, pH 7.4. To record K^+ currents, patch pipette was filled with solution consisting of 140 mM KCl, 1 mM $MgCl_2$, 3 mM Na_2ATP, 0.1 mM Na_2GTP, 0.1 mM EGTA, and 5 mM HEPES-KOH buffer, pH 7.2. To record the inwardly rectifying K^+ current, high-K^+, Ca^{2+}-free solution contained 130 mM KCl, 10 mM NaCl, 3 mM $MgCl_2$, 6 mM glucose, and 10 mM HEPES-KOH, pH 7.4. To record Ca^{2+} current, KCl inside the pipette solution was replaced with equimolar CsCl, and pH was adjusted to 7.2 with CsOH.

In the single-channel recording, high K^+-bathing solution contained 145 mM KCl, 0.53 mM $MgCl_2$, and 5 mM HEPES-KOH, pH 7.4 and pipette solution contained 145 mM KCl, 2 mM $MgCl_2$, and 5 mM HEPES-KOH, pH 7.2. The value of free Ca^{2+} concentration was calculated assuming the dissociation constant for EGTA and Ca^{2+} (at pH 7.2) at 0.1 μM (Portzehl et al. 1964).

RESULTS

Effect of Risperidone on Hyperpolarization-Activated Currents in GH_3 Cells

The whole-cell configuration of the patch-clamp technique was used to investigate the effect of risperidone on macroscopic ionic currents. When GH_3 cells were bathed in a high-K^+-Ca^{2+} free solution, a family of large inward current upon membrane hyperpolariza-

tion could be observed. Examples of the currents elicited by the 1-sec long clamp pulses to various membrane potentials from a holding potential of −10 mV are shown in Figure 1. Hyperpolarization voltage pulses were found to induce an instantaneous current followed by a voltage- and time-dependent activation of K^+ inward current. These inward currents were found to decay at potentials below −50 mV and the decay became faster with greater hyperpolarization (Wu et al. 1998). Within 1 min of exposing the cell to risperidone (1 μM), the amplitude of the hyperpolarization-elicited currents was significantly reduced. For example, when cells

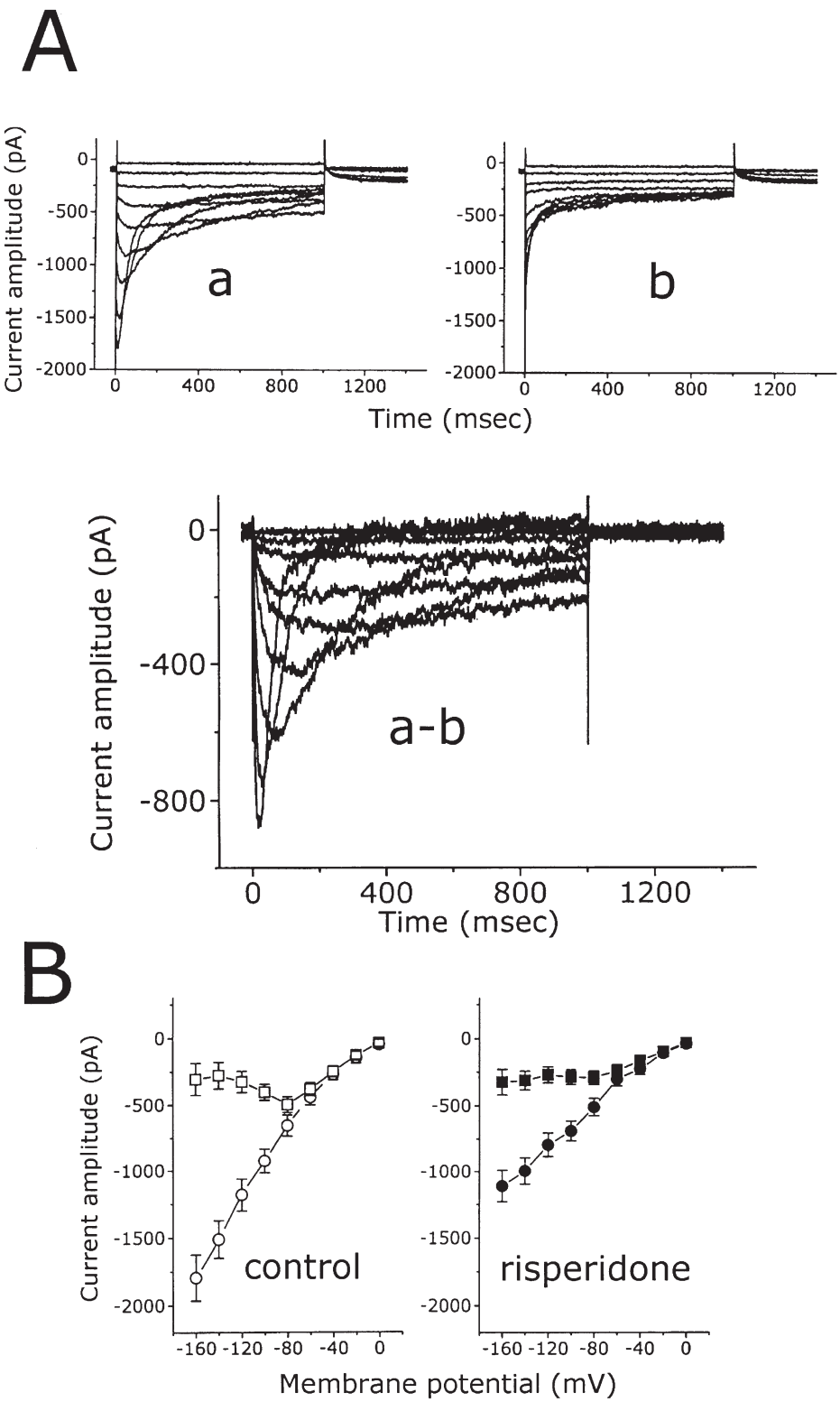

Figure 1. Inhibitory effect of risperidone on the current-voltage (I-V) relationships of the hyperpolarization-evoked currents in rat pituitary GH_3 cells. Cells were bathed in a high-K^+, Ca^{2+}-free solution containing tetrodotoxin (1 μM) and $CdCl_2$ (0.5 mM). **(A)** superimposed current traces obtained when a cell was held at the level of −10 mV and various voltage pulses ranging from 0 to −160 mV in 20-mV increments were applied. Current traces shown in Aa are controls and those in Ab were obtained 1 min after addition of risperidone (1 μM). The lower part of A shows the risperidone-sensitive inward currents (a–b). **(B)** averaged I-V relationships for initial (circle symbols) and steady-state (square symbols) components of ionic currents in the absence (left side) and presence (right side) of 1 μM risperidone. Each point represents the mean ± S.E.M. (n = 8–12).

were hyperpolarized from −10 to −120 mV, the presence of risperidone (1 μM) significantly decreased the amplitude of peak inward currents from 1804 ± 162 to 1115 ± 122 pA ($p < .05$, $n = 9$). This inhibitory effect was readily reversed on the removal of risperidone. However, there was no significant difference in current amplitudes taken at the end of the hyperpolarizing pulses from −10 to −120 mV (i.e., late sustained inward current) between the absence and presence of 1 μM risperidone [287 ± 82 pA ($n = 9$) versus 275 ± 64 pA ($n = 8$), $p > .05$].

When the difference of current traces between the presence and absence of risperidone (1 μM) was taken, the net change in membrane currents, i.e., the risperidone-sensitive component, was obtained. As shown in Figure 1, these risperidone-sensitive inward currents exhibited a voltage-dependence of activation and current decay similar to that of control currents, but with a much more pronounced inward rectification. However, the effect on membrane currents in the range of voltages near 0 mV was quite small. The presence of risperidone (1 μM) was found to significantly decrease the slope of the linear fit of current amplitudes to the voltages between −140 and −60 mV from 14.3 ± 4.1 to 8.8 ± 3.5 nS ($p < .05$, $n = 7$). These data suggest that an inwardly rectifying K^+ current ($I_{K(IR)}$) was present in GH_3 cells (Bauer et al. 1990; Wu et al. 1998) and that the blocking effect of risperidone was exerted primarily on the component of membrane currents that is responsible for an inward rectification.

The relationship between the concentration of risperidone and the percentage inhibition of $I_{K(IR)}$ is illustrated in Figure 2. The current amplitudes of $I_{K(IR)}$ in the presence of risperidone was compared with those after a subsequent application of E-4031 (10 μM). E-4031 was re-

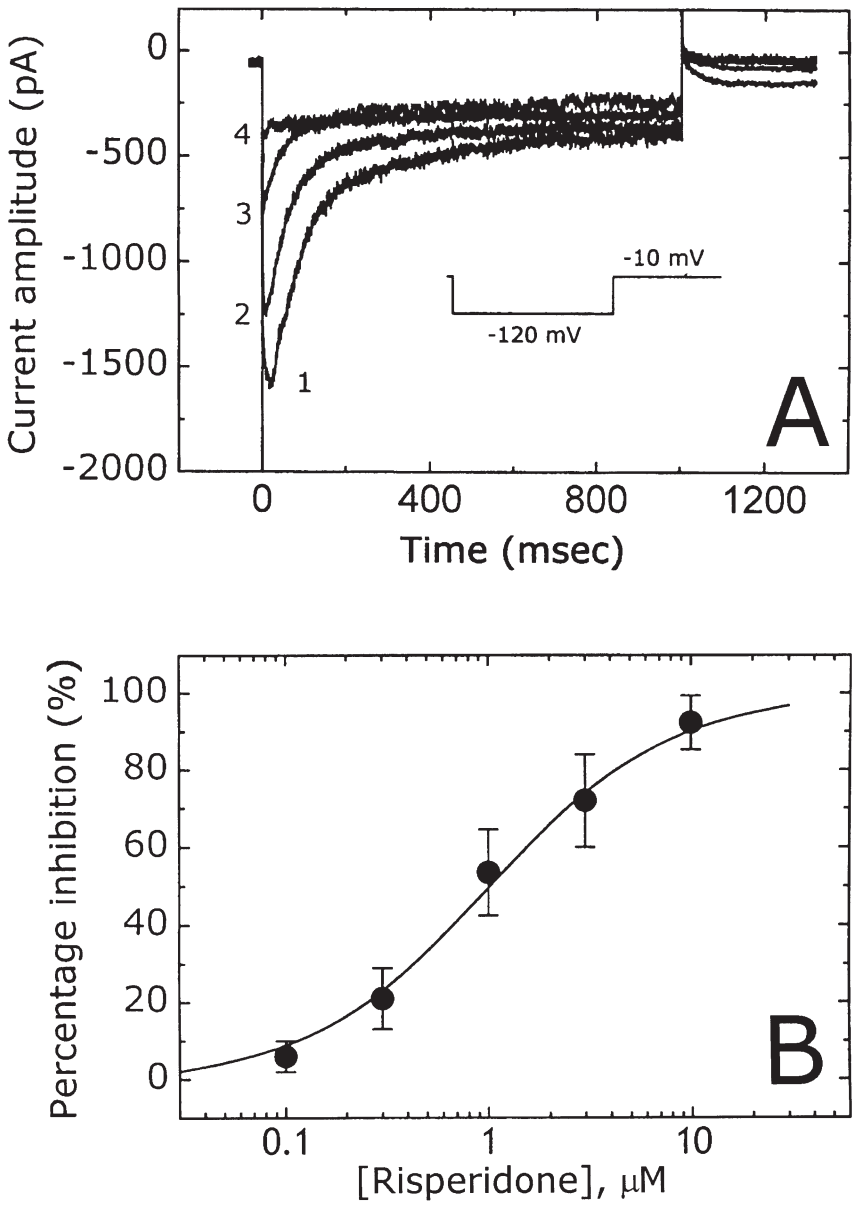

Figure 2. Concentration-dependent inhibition of $I_{K(IR)}$ by risperidone in GH_3 cells. **(A)** superimposed current traces obtained in the absence and presence of risperidone. Cells were bathed in a high-K^+ Ca^{2+}-free solution and the hyperpolarizing pulses from −10 to −120 mV were applied with a duration of 1 sec. Labeled 1 is the control, labeled 2 and 3 were obtained after the addition of 0.3 and 3 μM risperidone, respectively, and labeled 4 was after the addition of E-4031 (10 μM), but still in the presence of risperidone (3 μM). Inset indicates the voltage-clamp protocol. **(B)** Concentration-response relationship for risperidone-induced inhibition of $I_{K(IR)}$ (i.e., E-4031-sensitive current). Each point represents the mean ± S.E.M. ($n = 4$–8). The smooth line represents best fit to the Hill equation. The values for IC_{50}, maximally inhibited percentage of E-4031-sensitive current and Hill coefficient, were 1 μM, 99% and 1.1, respectively.

ported to be a selective blocker of $I_{K(IR)}$ (Weinsberg et al. 1997). Application of risperidone (0.1–10 μM) was found to suppress the amplitude of E-4031-sensitive currents in a concentration-dependent manner. The half-maximal concentration required for the inhibitory effect of risperidone on $I_{K(IR)}$ was 1 μM; and 10 μM risperidone almost completely suppressed the current amplitude.

Effect of Risperidone on the Recovery of Hyperpolarization-Elicited Transient Currents from Inactivation

The effect of risperidone on the recovery of hyperpolarization-elicited transient inward current from inactivation was studied with the double-pulse protocol. The recovery of transient inward current from inactivation at a holding potential of −10 mV was examined at different times with a test step (−120 mV for 1 sec) to determine the effect of risperidone on the rate of activation. This method was based on the fact that there is a rapid recovery from inactivation of $I_{K(IR)}$ as compared with its deactivation (Johnson et al. 1999). As depicted in Figure 3, in the control, the amplitude of peak inward current almost completely recovered from inactivation when the recovery time was 4 sec. The time course of recovery from inactivation in the control was fitted to a single exponential function with a time constant of 1.3 ± 0.5 sec ($n = 6$). However, in the presence of risperidone (3 μM), the recovery from inactivation was significantly prolonged with a time constant of 2.7 ± 0.8 sec ($p < .05$, $n = 5$).

After a 4-sec interval, the amplitude of peak inward current was found to completely recover from inactivation in the control; however, in the presence of risperidone, substantial block of peak inward current was still observed (Figure 3). Thus, the presence of risperidone caused a significant prolongation of the recovery from inactivation of hyperpolarization-elicited transient current in GH_3 cells. The results suggest that the activation of this current was slower in the presence of risperidone (Johnson et al. 1999).

Effect of Risperidone on the Deactivation of Hyperpolarization-Elicited Transient Currents

Deactivation kinetics in the absence and presence of risperidone was also assessed by measuring the rate of current decay (i.e., $1/\tau$) upon stepping to different voltages. In these experiments, the test pulses were preceded by a fixed, highly hyperpolarized prepulse (−120 mV for 20 msec) that would activate virtually all channels and uncouple the activation process from block. A range of the voltage pulses between −160 and −80 mV was then applied.

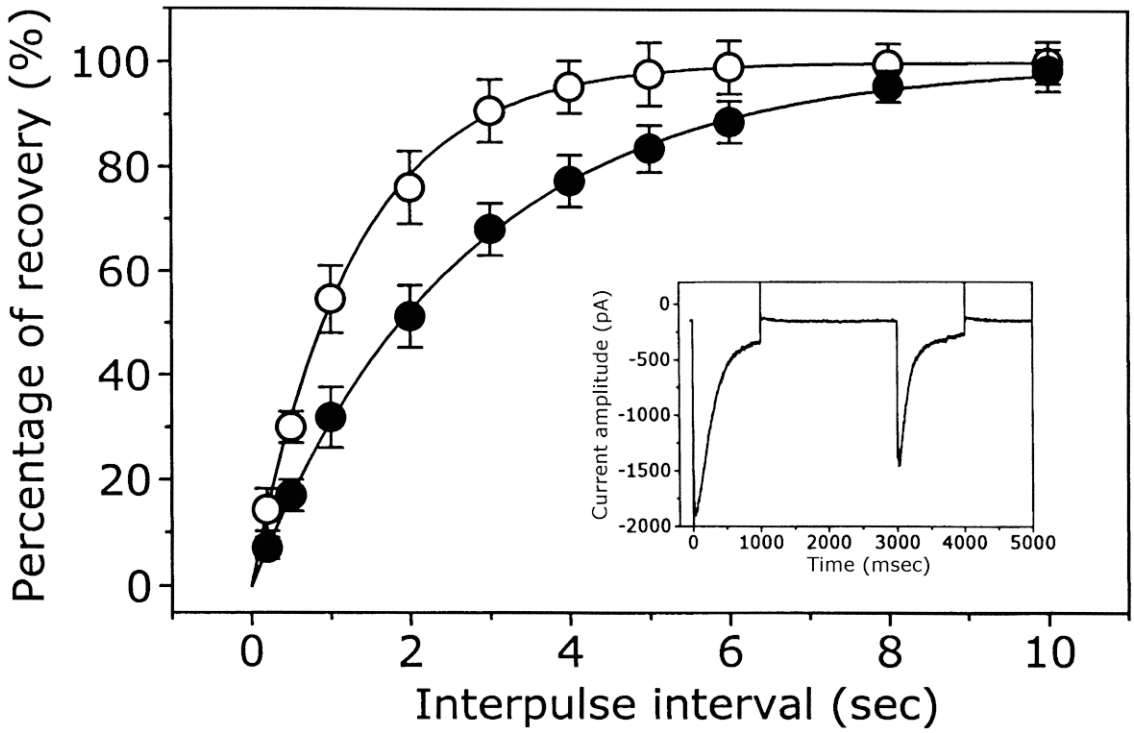

Figure 3. Effect of risperidone on the recovery of hyperpolarization-elicited transient currents from inactivation in GH_3 cells. Cells, bathed in high K^+-Ca^{2+} free solution, were hyperpolarized from −10 to −120 mV with a duration of 1 sec and various interpulse durations were applied. An example of current trace obtained by a two-pulse protocol is illustrated in inset. ○: control; ●: risperidone (3 μM). Note that after application of risperidone, the time course of recovery was slowed substantially.

After addition of risperidone to the bath, the rate of the current decay was assessed at each voltage pulse. The current decays in the absence and presence of risperidone (3 μM) were fitted with single exponential, and their reciprocal time constants ($1/\tau$) were then plotted against the test potential (Figure 4). The difference in the value of $1/\tau$ between the absence and presence of risperidone was found to be greater with less hyperpolarization. The results suggest that the presence of risperidone is capable of increasing the rate of channel deactivation at every voltage measured.

Comparison between Effect of Risperidone and Those of Various Neuroleptic Compounds on the Amplitude of $I_{K(IR)}$

The effects of various neuroleptic compounds, including haloperidol, thioridazine, clozapine, and metoclo-

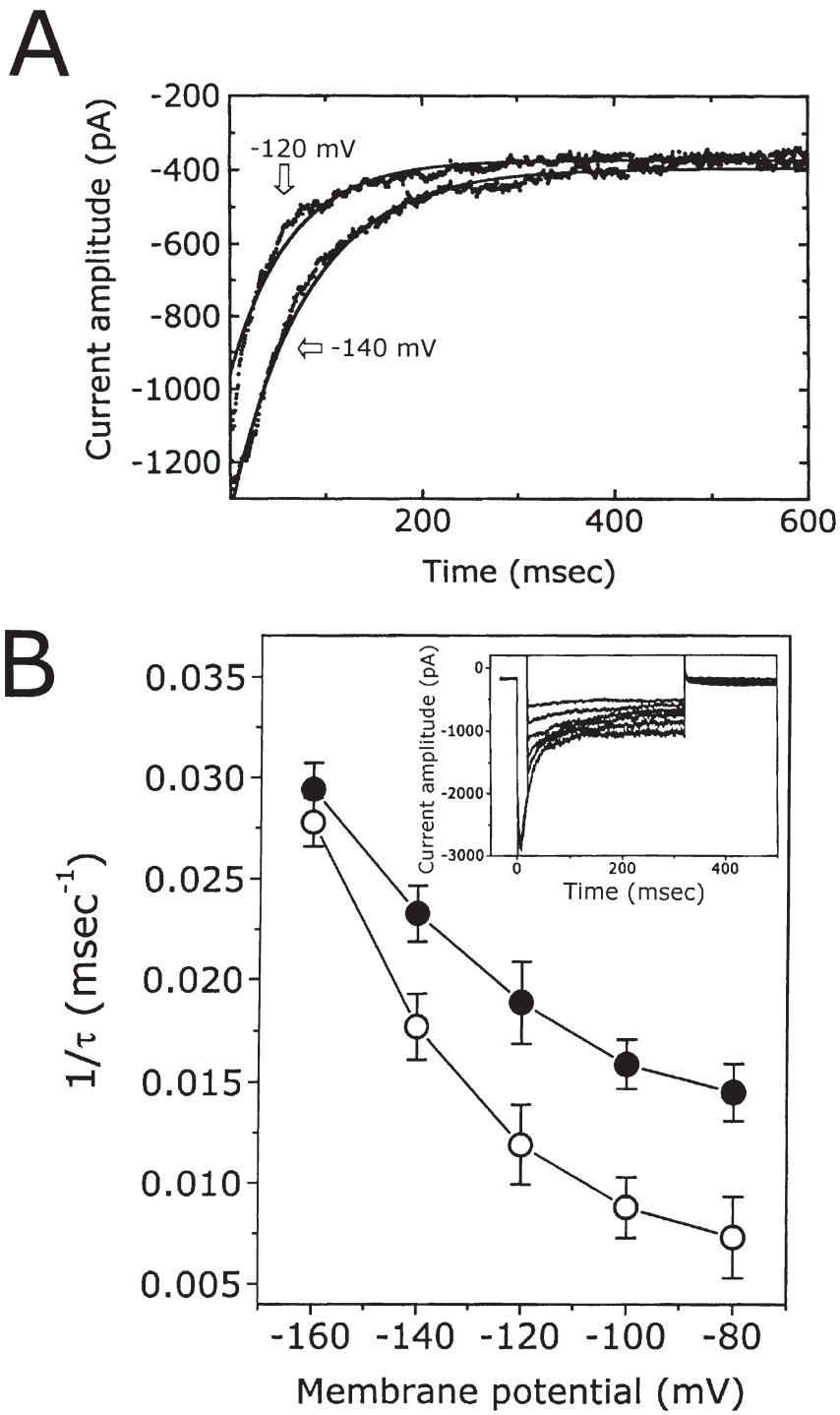

Figure 4. Risperidone effect on the deactivation of hyperpolarization-elicited transient currents in GH_3 cells. Cells were bathed in high K^+-Ca^{2+} free solution. The conditioning voltage pulses were hyperpolarized from −10 to −120 mV with a duration of 20 msec. After each conditioning pulse, various membrane potentials between −160 and −80 mV in 20-mV increments were delivered. In **A**, the time courses of current decay at the level of −120 and −140 mV in the control were well fitted by a single exponential with a value of 83 and 56 msec, respectively. In **B**, the reciprocal of the time constant obtained by single-exponential fit of current decay in the absence (○) and presence (●) of risperidone (3 μM) was plotted against the membrane voltage. The superimposed current traces obtained by a two-pulse protocol in the control are illustrated in inset.

pramide, on the amplitude of $I_{K(IR)}$ in these cells were examined and compared. As shown in Figure 5, both haloperidol (10 μM) and thioridazine (10 μM) can produce a significant inhibition on the amplitude of $I_{K(IR)}$. Clozapine (10 μM), another atypical psychotic agent, suppressed the amplitude of $I_{K(IR)}$ by about 20%. However, no significant change in the amplitude of $I_{K(IR)}$ was seen after addition of metoclopramide (10 μM). In addition, neither serotonin (10 μM) nor dopamine (10 μM) had any effect on $I_{K(IR)}$ (data not shown).

These results seem to indicate that the magnitude of the inhibitory effects on $I_{K(IR)}$ caused by these neuroleptic agents in GH_3 cells was not the same and was not associated with their blockade of serotonin or dopamine receptors.

Lack of Effect of Risperidone on Voltage-Dependent K^+ Outward Current (I_K) in GH_3 Cells

Because the neuroleptic agents were previously reported to suppress the amplitude of I_K in rat pheochromocytoma cells (Nakazawa et al. 1995), we considered whether risperidone might have any effect on this current. To address this issue, the experiments were conducted in GH_3 cells bathed in Ca^{2+}-free Tyrode's solution containing 1 μM tetrodotoxin and 0.5 mM $CdCl_2$. When the cells were depolarized from −60 mV to various membrane potentials ranging from −50 to +70 mV at 0.05 Hz, two components of voltage-dependent I_K were elicited, i.e., inactivating and non-inactivating components (Wu et al. 1999b). As shown in Figure 6, no significant effects of risperidone (10 μM) on both components of voltage-dependent I_K were observed. For example, the inactivating components estimated as the amplitude of peak outward current at the level of +70 mV between the absence and presence of 10 μM risperidone did not significantly differ [1544 ± 34 (n = 6) versus 1541 ± 32 pA (n = 5), $p > .05$].

The current amplitude measured at the end of voltage pulses (e.g., +50 mV) between the absence and presence of 10 μM risperidone also did not significantly differ [997 ± 21 (n = 6) versus 995 ± 18 pA (n = 5), $p > .05$]. Thus, the results presented here failed to demonstrate

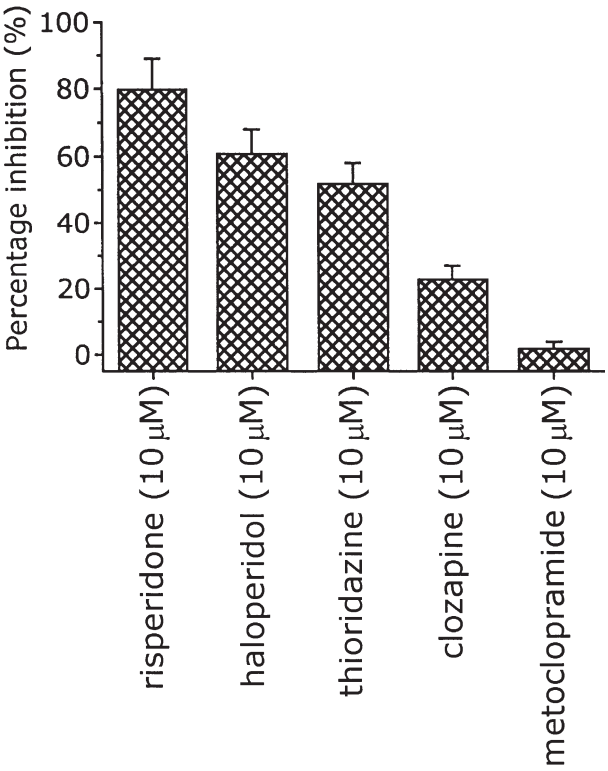

Figure 5. Comparison of the effect of risperidone and those of haloperidol, thioridazine, clozapine, and metoclopramide on the amplitude of $I_{K(IR)}$. Each cell was held at the level of −10 mV and the hyperpolarizing pulses to −120 mV (1 sec in duration) were delivered. Each bar represents the mean ± S.E.M. (n = 5–9).

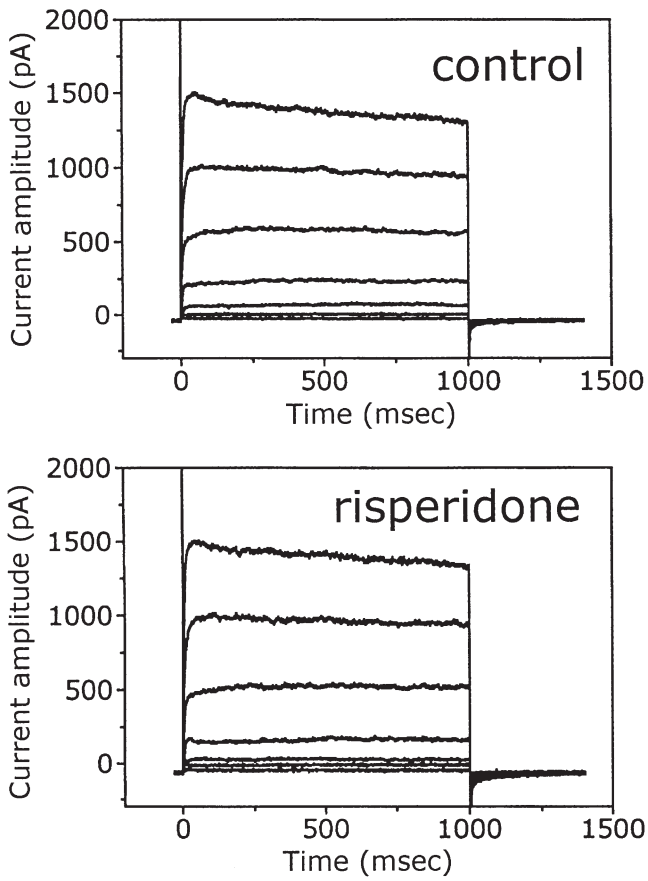

Figure 6. Lack of effect of risperidone on voltage-dependent K^+ outward currents (I_K) in GH_3 cells. Cells, bathed in Ca^{2+}-free Tyrode's solution containing tetrodotoxin (1 μM) and $CdCl_2$ (0.5 mM), were held at the level of −60 mV and the voltage pulses from −50 to −70 mV in 20-mV increments were applied. Superimposed current traces shown in upper part are controls, and those in lower part were recorded 2 min after addition of risperidone (10 μM).

that risperidone affected both inactivating and non-inactivating components of voltage-dependent I_K in GH_3 cells.

Comparison of the Effects of Risperidone and Haloperidol on Voltage-Dependent L-Type Ca^{2+} Current ($I_{Ca,L}$)

The effect of risperidone and haloperidol on the amplitude of $I_{Ca,L}$ was also assessed in this study. In these experiments, cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$ and the recording pipettes were filled with Cs^+-containing solution. As shown in Figure 7, the presence of risperidone (10 μM) did not affect the amplitude of $I_{Ca,L}$. For example, when cells were depolarized from −50 to 0 mV, there was no significant change in the amplitude of $I_{Ca,L}$ between the absence and presence of 10 μM risperidone [345 ± 15 (n = 6) versus 342 ± 14 pA (n = 6), $p > .05$].

On the other hand, when cells were exposed to haloperidol (10 μM), the amplitude of $I_{Ca,L}$ was significantly reduced to 225 ± 35 pA from a control of 332 ± 43 pA ($p < .05$, n = 5). However, neither haloperidol nor risperidone changed the overall I-V relationship of $I_{Ca,L}$. Thus, by comparison, haloperidol was more potent in suppressing $I_{Ca,L}$ than risperidone in GH_3 cells.

Inhibitory Effect of Risperidone on Large-Conductance Ca^{2+}-Activated K^+ (BK_{Ca}) Channels

The effect of risperiodone on the activity of BK_{Ca} channels was further examined. In these experiments, the single-channel recording with the inside-out configuration

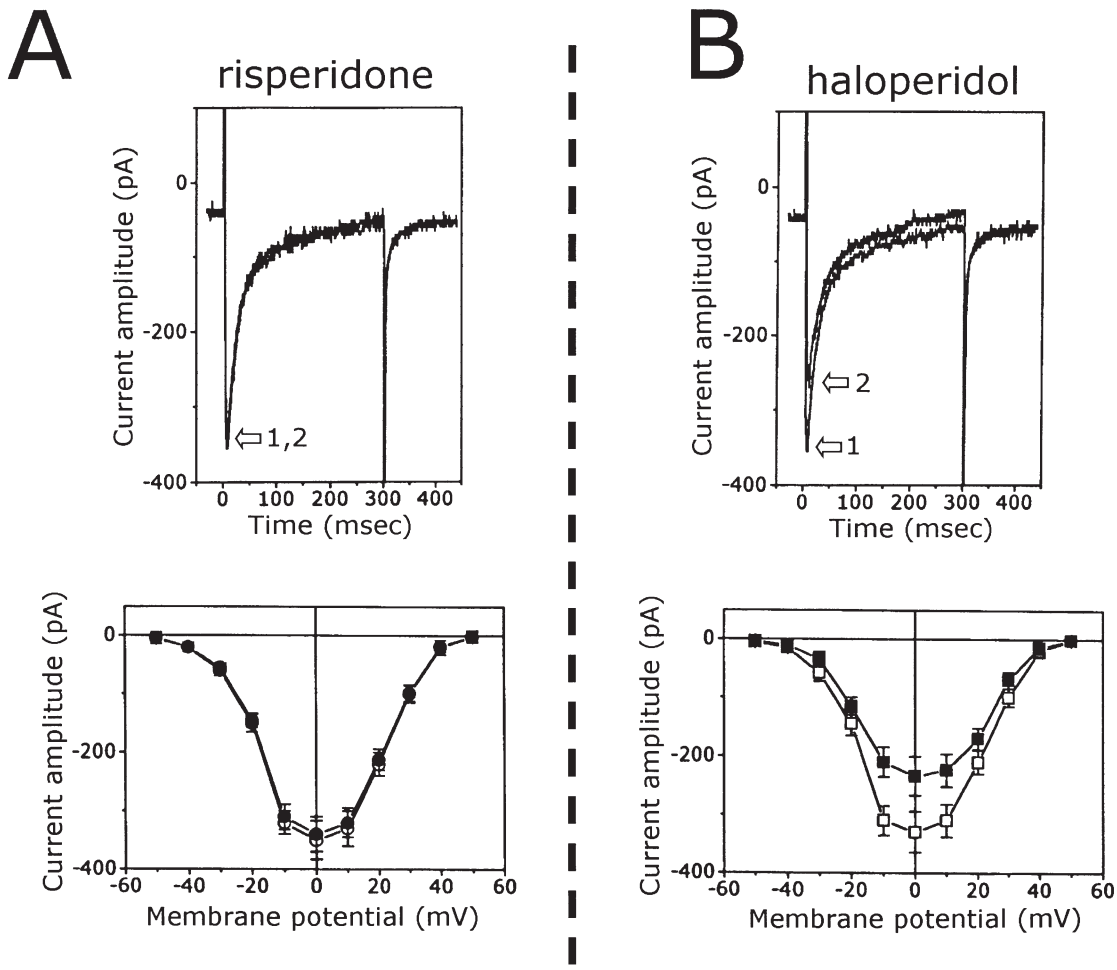

Figure 7. Comparison of the effect of risperidone **(A)** and haloperidol **(B)** on L-type Ca^{2+} inward currents ($I_{Ca,L}$) in GH_3 cells. Cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$, 1 μM tetrodotoxin, and 10 mM tetraethylammonium chloride. The recording pipette was filled with the Cs^+-containing solution. Cells were depolarized from −50 to 0 mV with a duration of 300 msec. Labeled 1 shown in the upper part of each panel indicates the control, and labeled 2 indicates the samples obtained after addition of 10 μM risperidone **(A)** or 10 μM haloperidol **(B)**. The I-V relationships of $I_{Ca,L}$ between the absence (open symbols) and presence (filled symbols) of 10 μM risperidone (10 μM) or haloperidol (10 μM) are illustrated in the lower part of each panel. Of note, haloperidol significantly suppressed $I_{Ca,L}$ without changing the I-V relationship of $I_{Ca,L}$ in GH_3 cells.

was performed in symmetrical K^+ (145 mM) solutions (Wu et al. 1999a). Bath medium contained 0.1 μM Ca^{2+} and holding potential was continuously set to +80 mV. The opening probability of the channel at the level of +80 mV in the control was found to be 0.022 ± 0.003 (n = 9).

When risperidone (3 μM) was applied to the bath, no significant change in the activity of BK_{Ca} channels was found [0.022 ± 0.003 (n =5) versus 0.022 ± 0.003 (n = 5), $p > .05$]. However, when the excised membrane patches were intracellularly exposed to risperidone at a concentration of 10 μM, the channel activity was significantly decreased (Figure 8). The addition of risperidone (10 μM) decreased the opening probability to 0.013 ± 0.003 ($p < 0.05$, n = 6).

On the other hand, there was no significant alteration in the single-channel conductance of BK_{Ca} channels between the absence and presence of 10 μM risperidone [183 ± 8 (n = 6) versus 181 ± 8 pS (n = 5), $p >$ .05]. In addition, haloperidol (10 μM) suppressed the channel activity by about 50% and penitrem A (1 μM) almost completely inhibited the channel activity (Figure 8B). Penitrem A was considered to be a potent blocker of BK_{Ca} channels (Knaus et al. 1994).

These results indicate that risperidone at a concentration of 10 μM did not change the single-channel conductance of BK_{Ca} channels, but was capable of significantly suppressing the channel activity that was sensitive to penitrem A.

Effect of Risperidone on the Firing of Action Potentials

In order to determine whether the effect of risperidone on membrane potential occurs in GH_3 cells, the experiments were conducted with a K^+-containing pipette solution and cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Under the current-clamp conditions, about 80% of cells exhibited the spontaneous firing of action potentials. This type of action potential was Ca^{2+}-dependent, because it can be abolished by further application of nimodipine (1 μM) or tetrandrine (5 μM), but not by tetrodotoxin (1 μM) (Kidoroko 1975; Wu and Li 1999). Nimodipine and tetrandrine are the inhibitors of $I_{Ca,L}$. Figure 9 illustrates the effect of risperidone on the firing of action potentials in GH_3 cells.

When risperidone (3 μM) was added to the bath, the membrane potential was significantly depolarized from −48 ± 6 to −38 ± 5 mV ($p < .05$, n = 8). The firing rate of action potential was increased to 0.44 ± 0.08 Hz from a control value of 0.12 ± 0.06 Hz ($p < .05$, n = 7). In addition, the presence of risperidone (3 μM) significantly increased action potential duration from 32 ± 10 to 42 ± 12 msec ($p < .05$, n = 6).

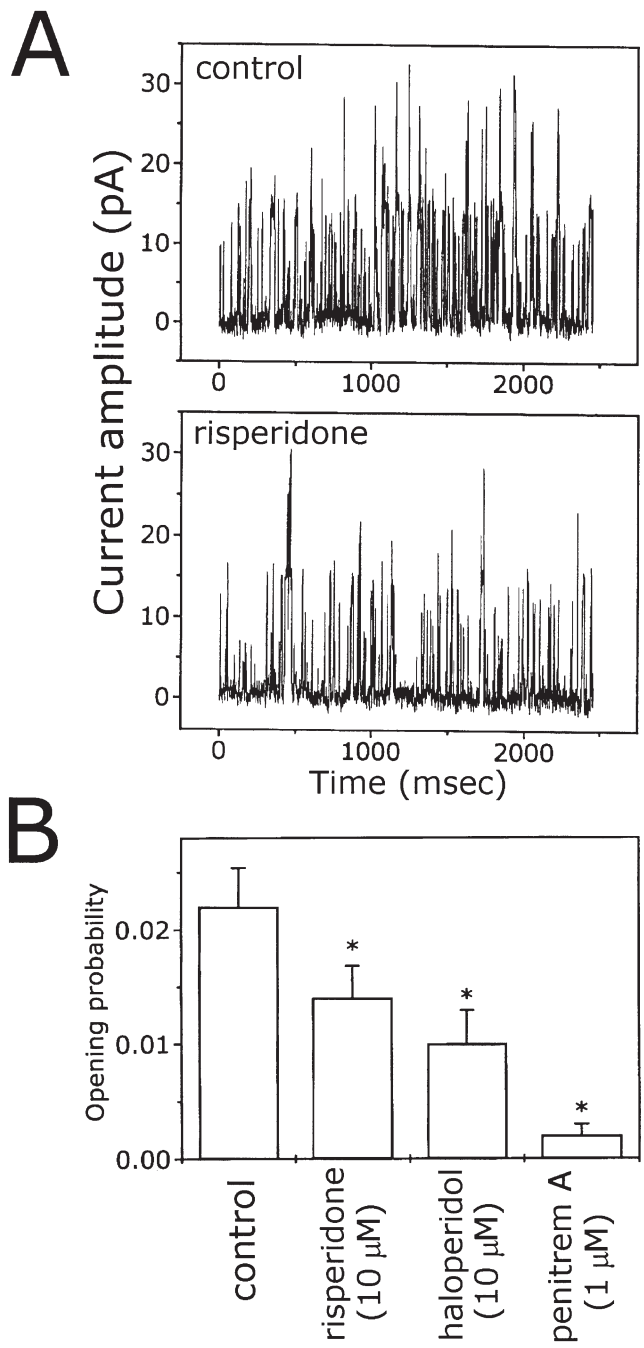

Figure 8. Effect of risperidone on the activity of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels in GH_3 cells. **(A)** Original current traces showing the activity of BK_{Ca} channels in the absence and presence of risperidone (10 μM). The inside-out configuration was performed and bath medium contained 0.1 μM Ca^{2+}. The holding potential was +80 mV. The detached membrane patch was intracellularly exposed to 10 μM risperidone. Upward deflections are due to the channel opening. **(B)** Bar graph showing the inhibitory effect of risperidone, haloperidol, and penitrem A on the activity of BK_{Ca} channels. Each point represents the mean ± S.E.M. (n = 5–9). $^*p < .05$ versus control.

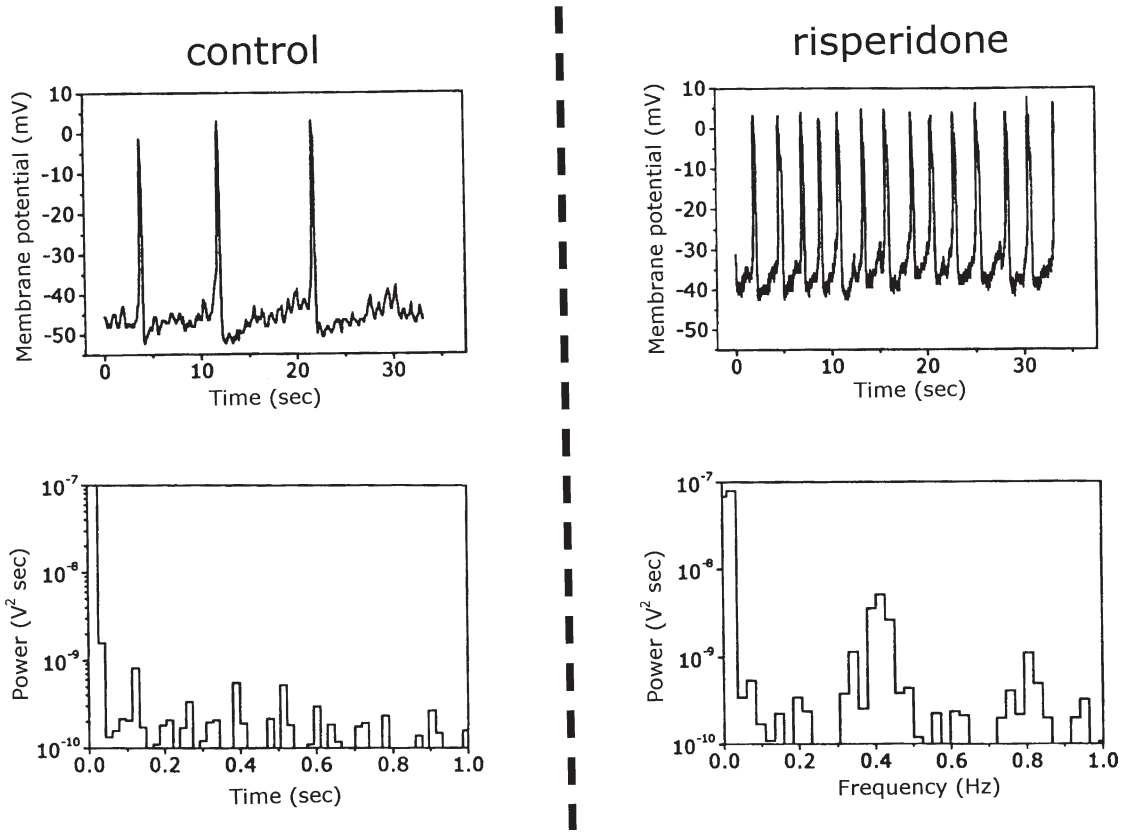

Figure 9. Effect of risperidone on the change in membrane potential of GH_3 cells. Cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The experiments were conducted in the current-clamp conditions. The upper part of the left panel is the control and the lower part of the right panel was obtained 2 min after addition of risperidone (3 μM). Risperidone (3 μM) caused membrane depolarization and induced an increase in the frequency of action potentials. Lower part in each panel shows the spectral pattern of firing action potentials in the absence and presence of 3 μM risperidone. Of note, when the cell was exposed to risperidone (3 μM), the repetitive firing was converted from an irregular (left panel) to a regular pattern (right panel).

Effect of Risperidone on the Discharge Pattern of Spontaneous Action Potentials in GH_3 Cells

The effect of risperidone on the firing pattern of action potentials was also investigated. To analyze the discharge pattern of spontaneous action potentials, the power spectral analyses of the change in membrane potential that can convert the time domain to the frequency domain were performed (Wu et al. 1996). The frequency of spontaneous action potentials in GH_3 cells was rather variable, ranging between 0.02 and 0.1 Hz.

As shown in Figure 9, the majority of GH_3 cells in the control were noted to exhibit the scattered power density in its spectrogram. These data indicate that there was an irregular pattern of the repetitive firing in control GH_3 cells. Interestingly, when cells were exposed to risperidone (3 μM), the frequency-domain analysis showed that the discharge pattern of these GH_3 cells exhibited a concentrated power density at approximately 0.42 Hz, with its subsequent harmonic components appearing at multiples of 0.42 Hz, e.g., 0.84 Hz. Similar results were found in seven different cells. Thus, exposure of GH_3 cells to risperidone produced an increase in the firing rate that was accompanied by the conversion from an irregular to a regular discharge pattern.

DISCUSSION

In the present study, we examined K^+ inward currents in GH_3 lactotrophs bathed in high-K^+ solution (isotonic KCl), as reported previously (Bauer et al. 1990; Wu et al. 1998). These K^+ currents evoked by membrane hyperpolarization were composed of two different components that can be distinguished on the basis of their sensitivity to risperidone or glyceryl nonivamide. One component is an inwardly-rectifying K^+ current ($I_{K(IR)}$) which exhibits the voltage-dependent activation and current decay during the hyperpolarizing pulses, whereas the other component is a sustained inward current which can be activated at potentials equal or positive to −50 mV. Consistent with a previous report (Schäfer et al. 1999), this study demonstrates that like E-4031, risperidone is capable of blocking

$I_{K(IR)}$ with an IC_{50} value of 1 μM, whereas the sustained inward component was not affected by risperidone.

$I_{K(IR)}$ is thought to play an important role in determining the resting membrane potential (−40 to −60 mV) and the electrical activity in GH_3 cells (Schwarz and Bauer 1999). In addition, because single GH_3 cells have a very high input resistance (1–5 GΩ), only small amounts of current are sufficient to cause the change in membrane polarization. Therefore, a block of outflow through this type of ionic channels would result in a depolarization and affect the firing rate of these cells (Chiesa et al. 1997).

In the present study, we found that risperidone slowed the rate of activation. This slowing in the rate of activation may be interpreted to be primarily due to the open-channel blockade, although the possibility that risperidone associates with one or more of the multiple deactivated states can not be ruled out. Our results, demonstrating that there was the difference in reciprocal time constants of current decays seen at different voltages, also suggest that risperidone may increase the rate of deactivation (Johnson et al. 1999). It is thus possible that the different level of membrane potential can influence its interaction with these currents. The sensitivity to risperidone in pituitary lactotrophs would, thus, be dependent on the preexisting level of resting membrane potential, the firing rate of action potential, or the concentration of risperidone used, assuming that the risperidone action in pituitary lactotrophs is similar to that on GH_3 cells shown here.

In the present study, we also demonstrated that in the current-clamp conditions, application of risperidone produced an increase in the firing rate of action potentials and a concomitant conversion from an irregular to a regular discharge pattern. These findings are consistent with an earlier work showing that in neuroblastoma cells, the *erg*-mediated K^+ current is responsible for the adaptation of spike frequency (Chiesa et al. 1997). However, it still needs to be further clarified whether the block of $I_{K(IR)}$ by risperidone is voltage-dependent, because it appears to be difficult to compare the kinetics of currents consisting predominantly of a transient inward component (i.e., $I_{K(IR)}$) in the control with those of currents primarily due to a sustained component in the presence of risperidone.

It should be mentioned that the K^+ outward currents upon depolarization when measured in normal Tyrode's solution containing 5.4 mM K^+ may comprise $I_{K(IR)}$ which, like *erg* current, is also voltage-dependent. If this were occurring, one would expect that risperidone might suppress voltage-dependent K^+ currents. However, this was not the case. The reason for this is currently unknown, but it appears to be due to the possibility that the relative contribution of $I_{K(IR)}$ to the measured outward currents was so small that a significant effect of risperidone could not be easily detected. Nevertheless, the present results showing that risperidone at a concentration less than 10 μM did not affect voltage-dependent Ca^{2+} inward or K^+ outward currents, suggest that risperidone is a relatively selective block of $I_{K(IR)}$ in GH_3 cells. This may, thus, enable us to asses the physiological function of $I_{K(IR)}$ by the evaluation of risperidone effects on membrane potential and electrical activity. Indeed, the findings demonstrating the concomitant increase of electrical activity and inhibition of $I_{K(IR)}$ caused by risperidone under the circumstances in which other regulated currents (e.g., Ca^{2+} and Ca^{2+}-activated K^+ currents) are not relatively altered, could be interpreted to be compatible with the notion that $I_{K(IR)}$ constitutes an important cell component regulating resting potential and hormonal secretion in these cells (Bauer et al. 1999; Schwarz and Bauer 1999).

Risperidone, like most neuroleptic drugs, has been recognized to interfere with the actions of dopamine as a neurotransmitter, particularly at D_2 and D_2-like receptors (Petty 1999; Vanhauwe et al. 1999). However, it seems unlikely that the effect of risperidone on $I_{K(IR)}$ observed in GH_3 cells is primarily due to its activity as an antagonist to dopamine D_2 receptors. The reason can be explained as follows: 1) neither dopamine nor metoclopramide affected the amplitude of $I_{K(IR)}$ in GH_3 cells, although haloperidol or thioridazine mimicked the risperidone-mediated inhibition of $I_{K(IR)}$. Metoclopramide was also reported to antagonize at dopamine receptor; 2) in GH_3 cells preincubated with dopamine (10 μM), the inhibitory effect of risperidone on $I_{K(IR)}$ was unaffected; and 3) risperidone at a higher concentration (10 μM) applied intracellularly also suppressed the activity of BK_{Ca} channels. Therefore, the carefulness may need to be made in ascribing the risperidone-mediated prolactin release to those caused by the blockade of dopamine receptors *in vivo* or *in vitro* (Bowden et al. 1992; Breier et al. 1999; Kim et al. 1999; Petty 1999; Carboni et al. 2000).

Because one of the physiological roles of the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels is thought to be cell repolarization following membrane depolarization and/or elevation of intracellular Ca^{2+}, the activity of these channels has been implicated in the control of neuroendocrine secretion (Wang et al. 1995; Kanyicska et al. 1997; Wu and Li 1999). In GH_3 cells, BK_{Ca} channels were found to be active at the level of resting membrane potential (Haug and Sand 1997).

Previous studies have also reported that neuroleptic agents, including risperidone, may cross cell membrane and affect mitochondrial respiratory chain (Maurer and Moller 1997; Balijepalli et al. 1999). Therefore, it is reasonable to speculate that the activity of BK_{Ca} channels in GH_3 cells can be intracellularly blocked by risperidone at a concentration greater than 10 μM, and that the risperidone-mediated inhibition of BK_{Ca} channels may contribute to the risperidone-mediated increase in

prolactin secretion. Moreover, because dopamine was found to stimulate the activity of BK_{Ca} channels in pituitary lactotrophs (Kanyicska et al. 1997), both blockade of dopamine receptors and direct inhibition of BK_{Ca} channels caused by risperidone may synergistically act to affect the functional activity of these cells *in vivo.*

In summary, the results of the present study show a significant block of $I_{K(IR)}$ by risperidone in GH_3 cells. This effect is presumably not mediated by the binding to dopamine receptors. The block of $I_{K(IR)}$ by risperidone will influence the cell excitability. Risperidone may be useful for isolating, mapping, and characterizing the inwardly rectifying K^+ channels. Interestingly, our results also imply that the risperidone-mediated stimulation of prolactin release could be partly, if not entirely, ascribed to the direct blockade of the inwardly rectifying K^+ and BK_{Ca} channels expressed in pituitary lactotrophs (Popli et al. 1998; Kleinberg et al. 1999; Wu et al. 1999b).

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