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Characterization of inhibition by haloperidol and chlorpromazine of a voltage-activated K^+ current in rat phaeochromocytoma cells

[1]Ken Nakazawa, Kanako Ito, Schuichi Koizumi, Yasuo Ohno & Kazuhide Inoue

Division of Pharmacology, National Institute Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

1 Inhibition by haloperidol and chlorpromazine of a voltage-activated K^+ current was characterized in rat phaeochromocytoma PC12 cells by use of whole-cell voltage-clamp techniques.

2 Haloperidol or chlorpromazine (1 and 10 μM) inhibited a K^+ current activated by a test potential of +20 mV applied from a holding potential of −60 mV. The K^+ current inhibition did not exhibit voltage-dependence when test potentials were changed between −10 and +40 mV or when holding potentials were changed between −120 and −60 mV.

3 Effects of compounds that are related to haloperidol and chlorpromazine in their pharmacological actions were examined. Fluspirilene (1 and 10 μM), an antipsychotic drug, inhibited the K^+ current, but pimozide (1 and 10 μM), another antipsychotic drug did not significantly inhibit the K^+ current. Sulpiride (1 or 10 μM), an antagonist of dopamine D_2 receptors, did not affect the K^+ current whereas (+)-SCH-23390 (10 μM), an antagonist of dopamine D_1 receptors, reduced the K^+ current. As for calmodulin antagonists, W-7 (100 μM), but not calmidazolium (1 μM), reduced the K^+ current.

4 The inhibition by haloperidol or chlorpromazine of the K^+ current was abolished when GTP in intracellular solution was replaced with GDPβS. Similarly, the inhibition by pimozide, fluspirilene, (+)-SCH-23390 or W-7 was abolished or attenuated in the presence of intracellular GDPβS. The inhibition by haloperidol or chlorpromazine was not prevented when cells were pretreated with pertussis toxin or when K-252a, an inhibitor of a variety of protein kinases, was included in the intracellular solution.

5 Haloperidol and chlorpromazine reduced a Ba^{2+} current permeating through Ca^{2+} channels. Inhibition by haloperidol or chlorpromazine of the Ba^{2+} current was not affected by GDPβS included in the intracellular solution.

6 It is concluded that haloperidol and chlorpromazine inhibit voltage-gated K^+ channels in PC12 cells by a mechanism involving GTP-binding proteins. The inhibition may not be related to their activity as antagonists of dopamine D_2 receptors or calmodulin antagonists.

Keywords: K^+ channels; antipsychotic drugs; dopamine receptor antagonists; calmodulin antagonists; GTP-binding proteins; Ca^{2+} channels

Introduction

Haloperidol and chlorpromazine are well-known antipsychotic drugs. Their therapeutic actions, namely, the alleviation of 'positive' symptoms of schizophrenia such as hallucinations and delusions have commonly been ascribed to antagonism at dopamine D_2 receptors (Seeman, 1980). Various antipsychotic drugs, including haloperidol and chlorpromazine are also reported to affect other cellular components. Chlorpromazine and structurally related compounds (e.g., Levin & Weiss, 1979; Hidaka *et al.*, 1980) and haloperidol (Levin & Weiss, 1979) antagonize calmodulin, a Ca^{2+}-binding protein that modulates various cellular functions. As for ion channels, inhibition by these compounds of voltage-gated Ca^{2+} channels has been well characterized. The block of Ca^{2+} channels by chlorpromazine and haloperidol was first demonstrated by Ogata *et al.* (1989) in mouse neuroblastoma N1E-115 cells. More recent investigations have shown that antipsychotic drugs inhibit L- (Fletcher *et al.*, 1994), T- (Enyeart *et al.*, 1992), N- (Fletcher *et al.*, 1994; Sah & Bean, 1994) and P-type Ca^{2+} channels (Sah & Bean, 1994)

Inhibition of K^+ channels by antipsychotic drugs has also been reported though it has been characterized less well than that of Ca^{2+} channels. Haloperidol and chlorpromazine blocked voltage-gated K^+ channels in N1E-115 cells (Ogata *et al.*, 1989). Chlorpromazine inhibited K^+ channels in rat sympathetic neurones (Wooltorton & Mathie, 1993) and rat alveolar epithelial cells (Jacobs & DeCoursey, 1990). Effects of other antipsychotic drugs were not examined in these papers. In addition, the cellular mechanisms underlying the K^+ channel inhibition have not been clarified.

In the present study, we have investigated the effects of haloperidol, chlorpromazine and related compounds on a voltage-activated K^+ current in rat phaeochromocytoma PC12 cells. Our results suggest that the inhibition of the K^+ current by haloperidol and chlorpromazine is not attributable to their activity as antagonists to dopamine D_2-receptors or calmodulin, and that GTP-binding proteins play an essential role in this inhibition.

Methods

PC12 cells (passage 55 to 70) were cultured according to Inoue & Kenimer (1988). Cells were plated on collagen-coated coverslips placed on the bottom of 35 mm polystyrene dishes. After culturing for 1 to 3 days at 37°C, cells were used for experiments. When cells were treated with pertussis toxin (PTX), it was added to the conditioned medium at a final concentration of 2 ng ml^{-1}, and the cells were incubated for 20 h at 37°C. Current recordings were made with conventional whole-cell voltage-clamp methods (Hamill *et al.*, 1981). The cells were bathed in an extracellular solution containing (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) 10, D-glucose 11.1 (pH was adjusted to 7.4 with NaOH). Tip re-

[1] Author for correspondence.

sistances of heat-polished patch pipettes ranged between 3 to 5 MΩ when the pipettes were filled with an intracellular solution containing (in mM): KCl 150, HEPES 10, ethyleneglycol-*bis*-(β-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA) 5, $MgCl_2$ 2.5, Na_2ATP 2, GTP 0.3 (pH 7.3 with KOH). Before application of drugs, K^+ current was activated by a 400 ms depolarizing step to +20 mV from a holding potential of −60 mV every 5 s for about 3 min. During this period, the K^+ current gradually declined to 70 to 80% of the initial amplitude, and reached a quasi-steady state. Application of drugs was made by superfusion at a constant flow rate of about 0.2 ml s^{-1}. When measuring Ba^{2+} currents permeating through voltage-gated Ca^{2+} channels, $CaCl_2$ and $MgCl_2$ were omitted from the extracellular solution and 20 mM $BaCl_2$ was added. KCl in the intracellular solution was replaced with CsCl and pH was adjusted with CsOH in this case. The amplitude of K^+ currents was determined as difference from a zero current level. As for Ba^{2+} currents, linear leak current estimated from current traces obtained with a hyperpolarizing step to −100 mV from a holding potential of −60 mV in each cell, and this component was subtracted to calculate the amplitude of the Ba^{2+} current. Experiments were performed at room temperature (about 25°C). Electrical signals were recorded with a patch clamp amplifier (Nihon Kohden CEZ-2400, Tokyo, Japan), filtered at 1 kHz and stored on magnetic tape for later analysis.

Drugs used are haloperidol (Serenance injection; Dainippon Pharmaceutical, Osaka, Japan), chlorpromazine hydrochloride (Wintamin injection; Shionogi, Osaka, Japan), pimozide, fluspirilene, (+)-SCH-23390 hydrochloride (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; Research Biochemicals, Natick, MA, U.S.A.), (-)-sulpiride (Research Biochemicals), W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulphonamide; Sigma, St. Louis, MO, U.S.A), calmidazolium (compound R24571; Sigma), ATP (adenosine 5'-triphosphate disodium salt; Sigma), GTP (guanosine triphosphate sodium salt; Sigma), GDPβS (guanosine 5'O-(2-thiodiphosphate) trilithium salt; Sigma), GTPγS (guanosine 5'-O-(3-thiotriphosphate tetralithium salt; Sigma) and K-252a (8R*,9S*,11S*)-(-)-9-hydroxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11 epoxy-1H,8H,11H-2,7b,11a-triazadibenzo(a,g)cycloocta (cde)trinden-1-one; Kyowa Hakko, Tokyo, Japan). Pertussis toxin was a kind gift from Dr J.G. Kenimer of FDA, U.S.A. Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan). Pimozide, fluspirilene, SCH-23390, sulpiride, W-7, calmidazolium and K-252a were first dissolved in dimethylsulphoxide (DMSO), and then diluted with extra- or intracellular solutions so that the final concentration of DMSO was less than 1%. In preliminary experiments, 1% DMSO did not affect K^+ or Ba^{2+} current. All the other drugs were dissolved directly in the solutions, or first dissolved in distilled water and then diluted in the solutions.

All the data in this paper are given as mean ± s.e.mean. Statistical difference was determined at the level of 0.05 ($P < 0.05$) using Student's t test or the paired t test.

Results

Haloperidol and chlorpromazine on K^+ current

When PC12 cells were held at −60 mV and a test potential of +20 mV was applied, an outward K^+ current was activated (Figure 1a) as previously reported for these cells (e.g., Hoshi & Aldrich, 1988; Nakazawa *et al.*, 1989). The current slowly

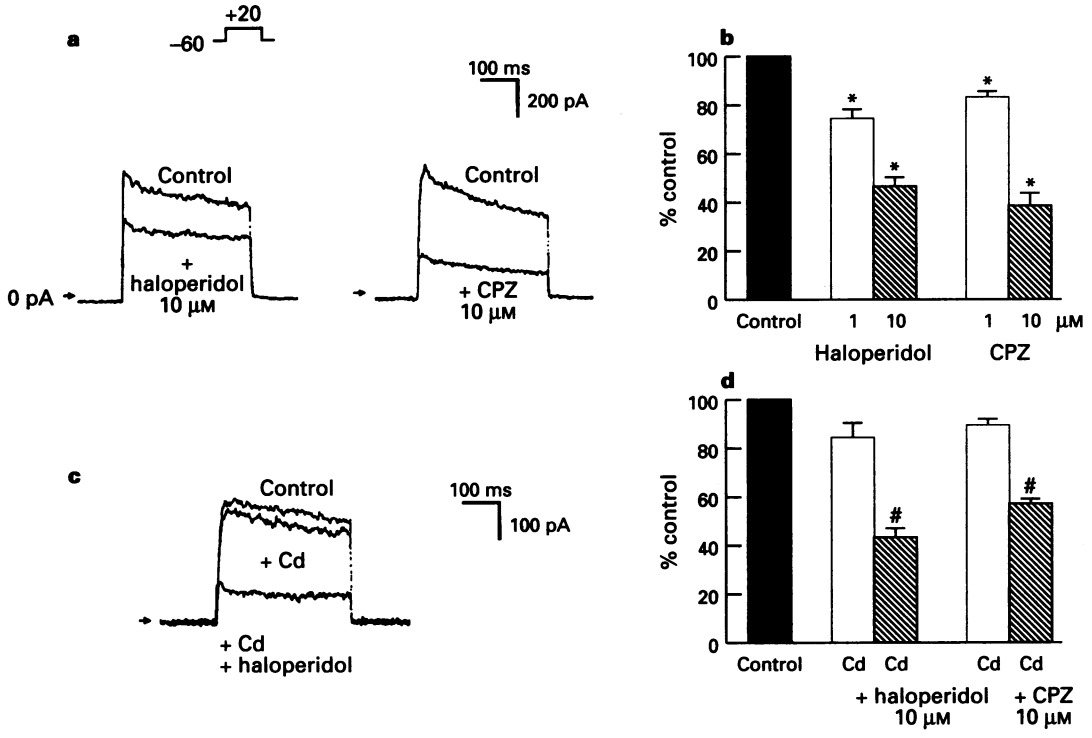

Figure 1 (a,b) Inhibition by haloperidol and chlorpromazine (CPZ) of a voltage-activated K^+ current. Cells were held at −60 mV, and a 400 ms depolarizing step to +20 mV was applied every 5 s. During repetitive depolarization, drugs were administered for 1 min. In (a) original traces of K^+ currents activated by the voltage-step in the absence and presence of 10 μM haloperidol (left) or chlorpromazine (right) were superimposed. An arrow indicates a zero current level. Peak K^+ current with drugs was normalized to that just before the drug application in individual cells, and summarized in (b). Data are mean ± s.e.mean obtained from 5 to 9 cells. Asterisks indicate significant difference from control current when compared by the paired t test using current amplitude before normalization (*$P < 0.05$). (c,d) K^+ current inhibition during the blockade of Ca^{2+} channels by Cd^{2+}. Cells were first exposed to 300 μM Cd^{2+} and then to 10 μM haloperidol or chlorpromazine in the presence of Cd^{2+}. Original current traces (c) and summarized data from 4 cells (d). The amplitude of K^+ current, activated as in (a), was normalized to that before addition of Cd^{2+} in individual cells, and shown as mean ± s.e.mean in (d). Significant difference from the current with Cd^{2+} when current amplitude before normalization were compared by the paired t test, #$P < 0.05$.

decayed during the depolarization: the current remaining after the end of a 400 ms pulse was usually 70 to 80% of the peak outward current. The peak outward current was largely reduced by 5 mM tetraethylammonium to 19.7±4.9% of control ($n=8$) or by 5 mM 4-aminopyridine to 28.6±5.3% of control ($n=8$). Judging from these properties, the current may be mainly mediated through a class of voltage-gated K^+ channels, which are dominant in undifferentiated PC12 cells and have been termed 'K_z-type' by Hoshi & Aldrich (1988).

Haloperidol and chlorpromazine inhibited the K^+ current (Figure 1a, b) at concentrations (1 and 10 μM) similar to those required for the block of K^+ currents by haloperidol in mouse neuroblastoma N1E-115 cells (Ogata *et al.*, 1989) or by chlorpromazine in guinea-pig ventricular cells (Klöckner & Isenberg, 1987), rat alveolar epithelial cells (Jacobs & DeCoursey, 1990) or rat sympathetic neurones (Wooltorton & Mathie, 1993). The inhibition was rapid in onset and reached steady-state within 30 s. The inhibition was partially reversible and the current recovered to 87.5±6.0% (10 μM haloperidol; $n=5$) or 81.4±3.5% (10 μM chlorpromazine; $n=4$) of control after a 1 min rinse with drug-free solution. The current component that did not recover appears to be irreversibly blocked because full recovery was not observed after a rinse of a longer period (up to 10 min). It has been reported that antipsychotic drugs including haloperidol and chlorpromazine inhibit various subclasses of voltage-gated Ca^{2+} channels (Ogata *et al.*, 1989; Enyeart *et al.*, 1992; Fletcher *et al.*, 1994 ; Sah & Bean, 1994). Haloperidol and chlorpromazine also inhibited Ba^{2+} currents permeating through Ca^{2+} channels in PC12 cells (Figure 6; see below). Thus, it is possible that these compounds do not directly inhibit K^+ channels, but their inhibition of Ca^{2+} channels results in reduction of Ca^{2+}-dependent K^+ channels, producing apparent reduction in the voltage-activated K^+ current. However, this does not seem to be the case in PC12 cells because (1) the K^+ current was reduced by only about 10 to 20% in the presence of 300 μM Cd^{2+} (Figure 1c, d), which is sufficient to block Ca^{2+} channels completely in these cells (Nakazawa *et al.*, 1991), and (2) haloperidol (10 μM) or chlorpromazine (10 μM) significantly reduced the K^+ current in the presence of 300 μM Cd^{2+} (Figure 1c, d). Chlorpromazine (10 μM) was less effective in reducing the K^+ current in the presence of 300 μM Cd^{2+} (Figure 1d). We cannot conclude at present whether this arises from inhibition by chlorpromazine of the current component mediated by Ca^{2+}-influx or other unknown actions of Cd^{2+} such as modification of the sensitivity of the Ca^{2+}-independent K^+ channels to chlorpromazine.

Figure 2a illustrates the K^+ current obtained with various test potentials between −10 and +40 mV. Haloperidol (10 μM) or chlorpromazine (10 μM) inhibited the current measured at all these potentials (Figure 2b, c). The inhibition by these compounds was not voltage-dependent, judging from a plot of the current fraction remaining after block against test potentials (Figure 2d). Wooltorton & Mathie (1993) reported that a transient K^+ current was elicited by depolarizing steps in rat sympathetic neurones only when the steps were applied from holding potentials as negative as −120 mV, and this current was less sensitive than a K^+ current activated from −70 mV. Figure 3a shows K^+ currents in PC12 cells obtained with voltage-protocols similar to those utilized by Wooltorton & Mathie (1993). The K^+ current activated from −120 mV was larger than that from −60 mV, and the current decay during the depolarization was slowed (Figure 3a). The result suggests that holding at −60 mV clearly does not remove all inactivation of the current under study. Unlike rat sympathetic neurones, a transient current component was not observed when the K^+ current was activated from −120 mV (Figure 3a, left). The currents activated from −60 and −120 mV were similarly inhibited by 10 μM haloperidol (Figure 3a, right). The magnitude of the inhibition by 10 μM haloperidol or 10 μM chlorpromazine was scarcely affected by changing prepulses between −120 and −60 mV (Figure 3b).

In addition to the inhibition of the K^+ current, a slight inward shift in holding current at −60 mV was observed during application of 10 μM haloperidol or chlorpromazine in some cells (e.g. Figure 2a). This current may be due to the enhancement of an inward current component because the shift was augmented by hyperpolarization (see Figure 3a, right for example). This component may produce a small underestimate of the inhibitory effect on the K^+ current at positive potentials. However, it may not be serious because the shift was maximally −50 pA at −60 mV and the absolute shift may have been even smaller around +20 mV.

Compounds related to haloperidol and chlorpromazine

Effects of various compounds which are related to haloperidol and chlorpromazine were examined (Figure 4). Haloperidol and chlorpromazine are well-known antipsychotic drugs. Pimozide (1 and 10 μM), another antipsychotic drug, did not significantly reduce the K^+ current whereas fluspirilene (1 and 10 μM), which is structurally similar to pimozide and also known as an antipsychotic drug, inhibited the K^+ current. Haloperidol and chlorpromazine exhibit an antagonist action on dopamine receptors (Seeman, 1980). SCH-23393 (1 and 10 μM), a selective dopamine D_1-receptor antagonist, significantly reduced the K^+ current. On the other hand, sulpiride (1 and 10 μM), a selective dopamine D_2-receptor antagonist, failed to inhibit the K^+ current. Another pharmacological property shared by haloperidol and chlorpromazine is antagonism of calmodulin (Levin & Weiss, 1979; Hidaka *et al.*, 1980). Two calmodulin antagonists, W-7 (Hidaka *et al.*, 1980) and calmidazolium, (Gietzen *et al.*, 1981) were tested on the K^+ current at concentrations that block calmodulin-dependent responses (10 and 100 μM for W-7 and 1 and 10 μM for calmidazolium). W-7 did not affect the K^+ current at 10 μM, but it significantly reduced the K^+ current at 100 μM. Calmidazolium did not block the K^+ current at 1 μM (Figure 4). At 10 μM, effects of calmidazolium were not consistent: in 5 cells tested, the K^+ current was reduced by about 40% in 2 cells, the current was increased by 80% in 1 cell, and the current measurement was not possible because of induction of an irreversible large inward current (>1 nA at −60 mV) in the remaining 3 cells (not shown).

Involvement of GTP-binding proteins

Involvement of GTP-binding proteins was tested by including 2 mM GDPβS, an inhibitor of these proteins, in the intracellular solution instead of GTP. GDPβS prevented the inhibition by 10 μM haloperidol or chlorpromazine of the K^+ current: the current became almost insensitive to these compounds under these conditions (Figure 5a, b). GDPβS also prevented the inhibition by 10 μM SCH-23393 or 100 μM W-7 (Figure 5b). The reduction by 10 μM fluspirilene of the K^+ current also appeared to be attenuated by GDPβS though the difference was not statistically significant (Figure 5b). Effects of GTPγS, a GTP analogue that persistently binds to GTP-binding protein, were examined (Figure 5c). GTPγS (500 μM), included in intracellular solution instead of GTP, did not affect the reduction by 1 μM haloperidol or chlorpromazine of the K^+ current, but it significantly attenuated the reduction by 10 μM haloperidol or chlorpromazine. Pertussis toxin (PTX) is known to block subclasses of GTP-binding proteins and thereby suppress ion channel modulations mediated by these proteins (Hille, 1992; 1994). PC12 cells were pretreated with PTX according to Inoue & Kenimer (1988). Following this procedure, a 41 kDa protein, presumably a PTX-sensitive GTP-binding protein, was almost completely ADP-ribosylated (Inoue & Kenimer, 1988), and the modulations by adenosine of extracellular ATP-evoked responses (Inoue *et al.*, 1994; Koizumi *et al.*, 1994) or the reduction by 5-hydroxytryptamine of the ATP-evoked responses (Koizumi *et al.*, 1995) was abolished in these cells. The pretreatment of PC12 cells with PTX, however, did not prevent the inhibition by 10 μM haloperidol or chlorpromazine of the K^+ current (Figure 5d).

We also tested the involvement of protein phosphorylation in the inhibition of the K^+ current by including 1 μM K-252a, an inhibitor of a variety of protein kinases (Kase *et al.*, 1987), in the intracellular solution. In this case, ATP was omitted from the intracellular solution. Under these conditions, the facilitation by dopamine of an inward current activated by extracellular ATP (Nakazawa *et al.*, 1993) or the inhibition by adenosine of the ATP-activated current (Inoue *et al.*, 1994) was abolished as in our previous experiments. In the presence of 1 μM K-252a, the K^+ current was also significantly reduced to $60.3 \pm 4.3\%$ (10 μM haloperidol; $n=3$) or $60.0 \pm 4.4\%$ of control (10 μM chlorpromazine; $n=3$), respectively. The magnitude of the current reduction by chlorpromazine, but not that by haloperidol, was significantly smaller (Student's t test, $P<0.05$) in the cells loaded with 1 μM K-252a than in the control cells (Figure 1b). These results suggest that protein phosphorylation does not play an essential role in the current reduction though it may play a minor role.

GDPβS, GTPγS or K-252a included in the intracellular solution did not effect the initial decrease of the K^+ current before application of drugs (see Methods): the amplitude of the K^+ current in the cells dialysed with these compounds declined to 70 to 80% of the initial amplitude during this period, as was the case in cells dialysed with a standard intracellular solution.

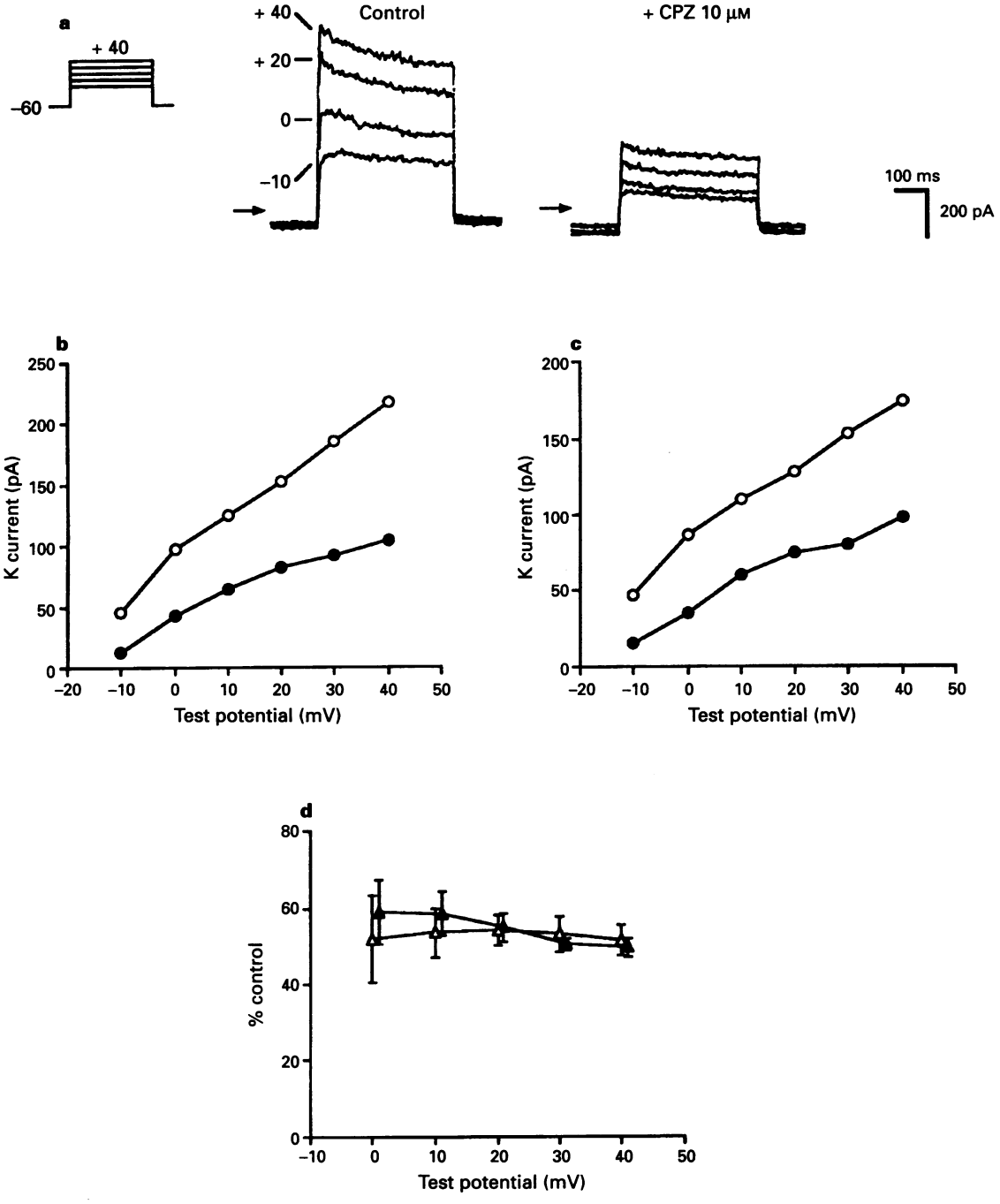

Figure 2 Inhibition by haloperidol (10 μM) and chlorpromazine (CPZ; 10 μM) of K^+ current at various test potentials. Cells were held at -60 mV and 400 ms voltage-steps in a 10 mV increment were applied every 5 s. (a), (b) and (c) were obtained from three different cells. (a) K^+ current at -10, 0, $+20$ and $+40$ mV and its inhibition by chlorpromazine. Arrows indicate a zero current level. (b, c) Current-voltage relationship for peak K^+ current in the absence (○) and presence of haloperidol (b) or chlorpromazine (c) (●). Current amplitude was plotted against test potentials. (d) Current fraction remaining after block by haloperidol (△) or chlorpromazine (▲) at various test potentials. Peak K^+ current with drugs were normalized to that before the drug-application at each test potential in individual cells. Data are mean ± s.e.mean from 4 cells.

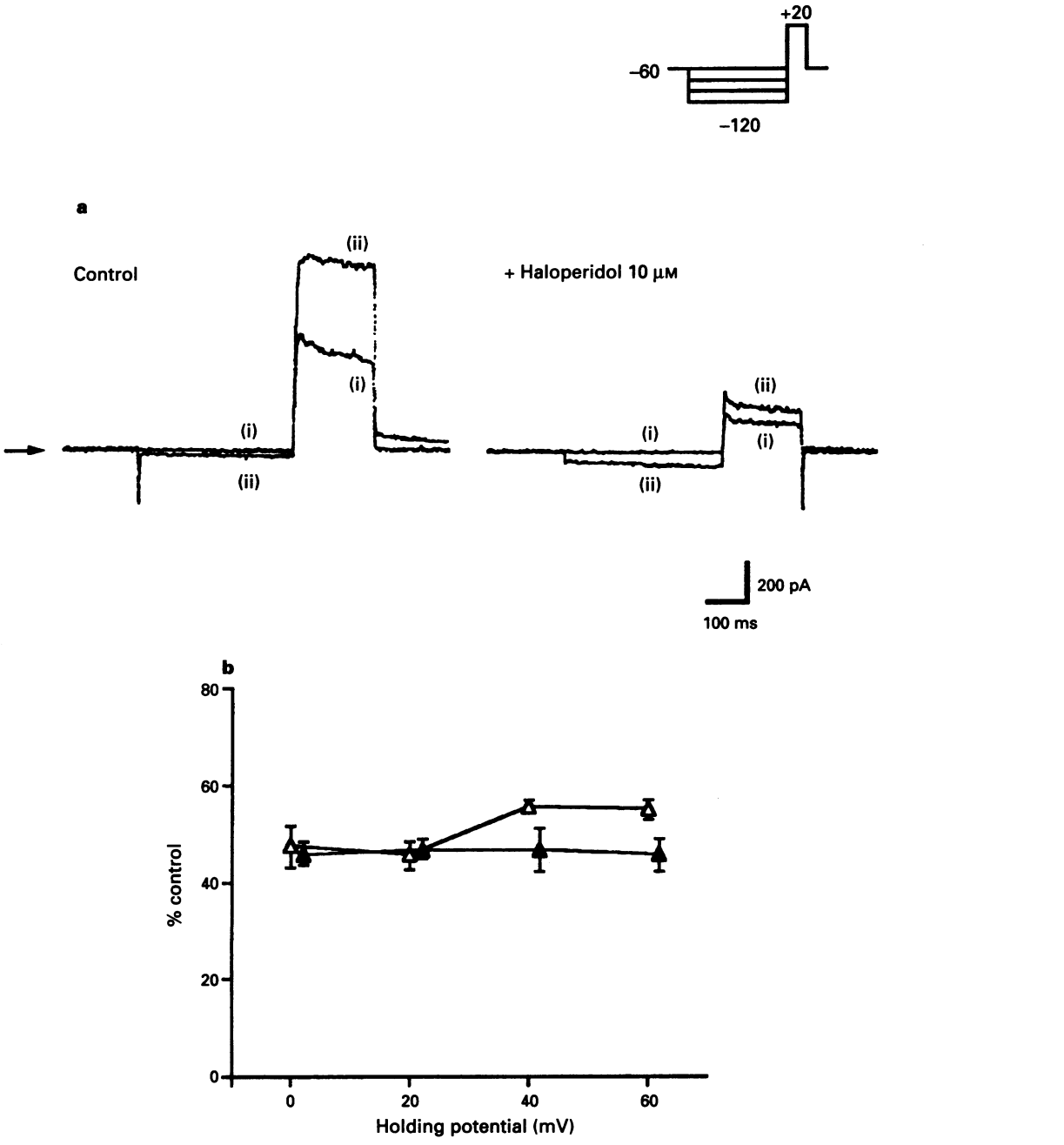

Figure 3 Inhibition by haloperidol (10 μM) and chlorpromazine (10 μM) of K^+ current activated from various holding potentials. Before K^+ current was activated by a 200 ms depolarizing step to +20 mV, cells were held at various potentials by applying a 300 ms prepulse from −60 mV. (a) K^+ currents with and without a prepulse of −120 mV in a cell (left), and their suppression by haloperidol (right). Current traces without the prepulse (i) and with the prepulse (ii) were superimposed. An arrow indicates a zero current level. (b) Current fraction remaining after block by haloperidol (△) or chlorpromazine (CPZ; ▲) obtained with various holding potentials. Peak K^+ currents with drugs were normalized to that before the drug-application in individual cells. Data are mean ± s.e.mean from 4 cells.

Haloperidol and chlorpromazine on Ba^{2+} current permeating through Ca^{2+} channels

Effects of haloperidol and chlorpromazine on voltage-gated Ca^{2+} channels were compared in the absence and presence of intracellular GDPβS to examine involvement of GTP-binding proteins. When cells were bathed in extracellular solution containing 20 mM Ba^{2+} instead of 1.8 mM Ca^{2+}, an inward current was activated by depolarizing voltage-steps. The inward current is carried by Ba^{2+} mostly through L-type Ca^{2+} channels, judging from its sensitivity to dihydropyridine Ca^{2+} channel antagonists as previously shown for undifferentiated PC12 cells (Plummer *et al.*, 1989; Nakazawa *et al.*, 1991). Haloperidol (10 μM) and chlorpromazine (10 μM) inhibited the Ba^{2+} current by about 50% in the cells loaded with standard intracellular solution containing 300 μM GTP (Figure 6b). The magnitude of the inhibition was in a range expected from previous work concerning block of ionic currents through L-type Ca^{2+} channels (Klöckner & Isenberg, 1987; Fletcher *et al.*, 1994) or replacement of [^{3}H]-nitrendipine binding (Gould *et al.*, 1983) by these compounds in other types of cells. The inhibition by haloperidol or chlorpromazine was not affected by 2 mM GDPβS included in intracellular solution instead of GTP (Figure 6).

Discussion

Haloperidol and chlorpromazine inhibited a voltage-activated K^+ current in PC12 cells. The blockade by haloperidol (Ogata

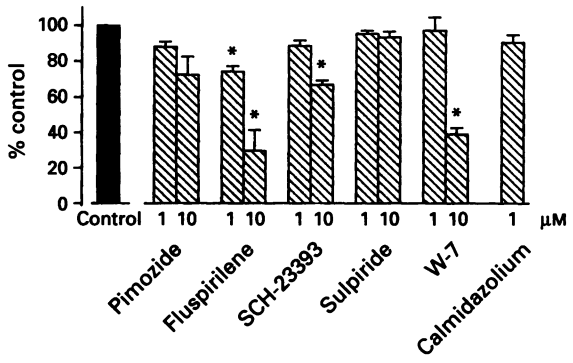

Figure 4 Effects of various compounds related to haloperidol and chlorpromazine on K^+ current. Data were obtained and shown in Figure 1. Each column represents mean ± s.e.mean from 4 to 6 cells.

et al., 1989) or chlorpromazine (Klöckner & Isenberg, 1987; Ogata *et al.*, 1989; Jacobs & DeCoursey, 1990; Wooltorton & Mathie, 1993) of K^+ currents has already been reported in other types of cells. The K^+ current in this study has some similarities to the currents previously described in kinetics, voltage-dependence and the concentrations of haloperidol or chlorpromazine required for the current inhibition. We have further characterized the inhibition by haloperidol and chlorpromazine of the K^+ current and clarified the following points: (1) some compounds pharmacologically related to haloperidol and chlorpromazine, such as fluspirilene, mimic the inhibition, but other related compounds, such as sulpiride, did not mimic it (Figure 4); (2) GTP-binding proteins may be essential for the inhibition, judging from the prevention by GDPβS of the inhibition (Figure 5).

Antipsychotic drugs including haloperidol and chlorpromazine antagonize dopamine at dopamine D_2 receptors and this is generally believed to be relevant to their therapeutic actions (Seeman, 1980). However, the inhibition of the K^+ current may not be attributed to antagonism at D_2 receptors because sulpiride, a selective D_2 receptor antagonist, or pimozide, an antipsychotic drug that also antagonizes at these receptors, did not inhibit the K^+ current. Antagonism to calmodulin, which both haloperidol and chlorpromazine exhibit (Levin & Weiss, 1979; Hidaka *et al.*, 1980), may not be related to the K^+ current inhibition, either, because calmidazolium failed to reduce the current (Figure 4). Wooltorton & Mathie (1993) have demonstrated that imipramine, a tricyclic antidepressant, and four structurally related compounds including chlorpromazine inhibit voltage-gated K^+ channels. The results in the present study, combined with the block by haloperidol of the K^+ channels in N1E-115 cells (Ogata *et al.*, 1989), indicate that compounds with molecular structures different

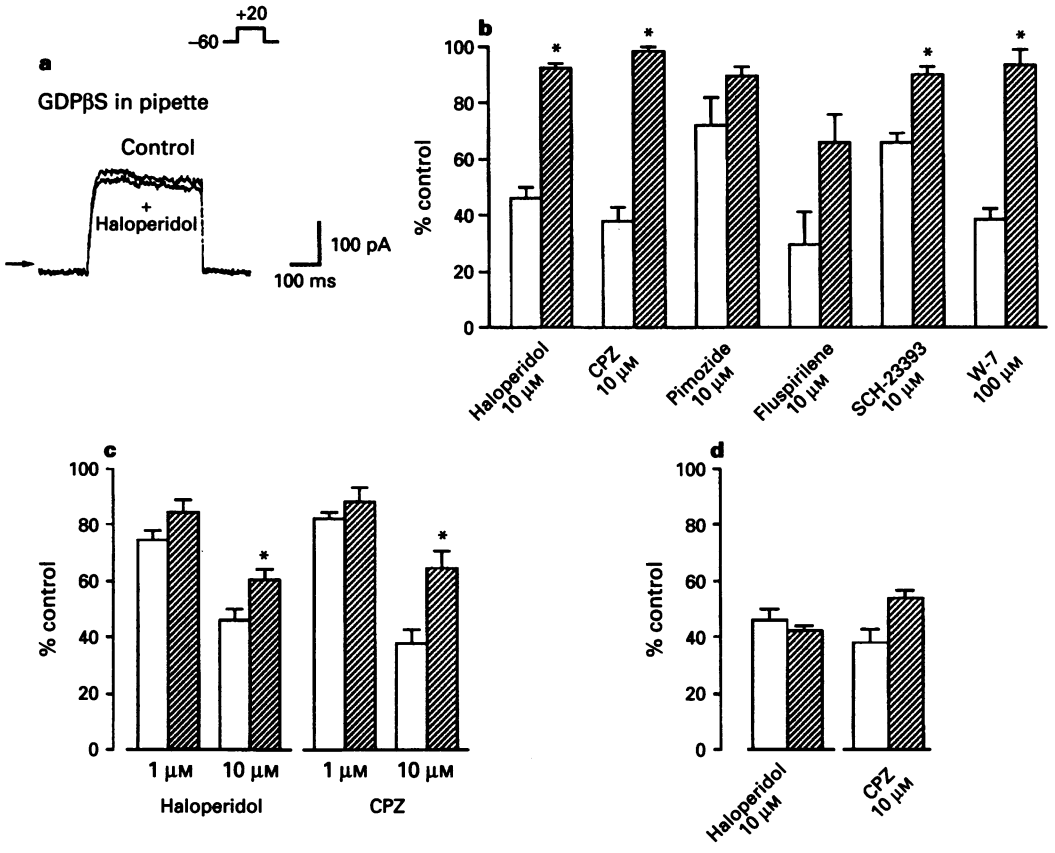

Figure 5 Tests for involvement of GTP-binding proteins in the K^+ current inhibition. Data were obtained and shown as in Figure 1. (a) K^+ currents in the absence and presence of 10 μM haloperidol in a cell loaded with 2 mM GDPβS instead of GTP. An arrow indicates a zero current level. (b) Attenuation by GDPβS of the K^+ current inhibition by haloperidol, chlorpromazine (CPZ) and related compounds. The current remaining after the inhibition with standard intracellular solution (open columns) and that with intracellular solution containing GDPβS (hatched columns) were compared for each compound. Data were obtained from 4 to 9 cells tested. Asterisks indicate significant difference from the inhibition with the standard solution determined by Student's *t* test (*$P < 0.05$). (c) Influence by GTPγS (500 μM) included in intracellular solution of K^+ current inhibition by haloperidol and chlorpromazine. Data were obtained from 4 to 9 cells. The K^+ current inhibition was compared between the cells loaded with standard solution (open columns) and those with GTPγS-containing solution (hatched columns) as shown in (b). (d) Failure of pertussis toxin (PTX) to prevent K^+ current inhibition. The inhibition in the cells treated with PTX (hatched columns) was compared with that in untreated cells (open columns). Data were obtained from 4 to 9 cells.

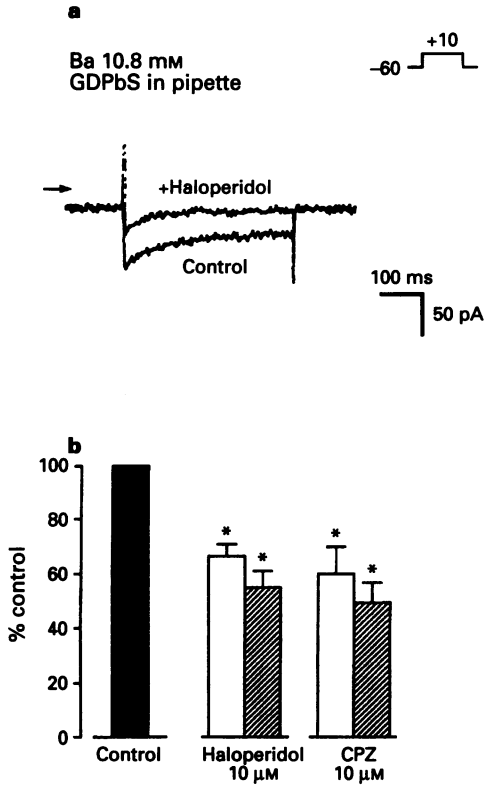

Figure 6 Ba^{2+} current permeating through voltage-gated Ca^{2+} channels and its inhibition by haloperidol and chlorpromazine (CPZ). Cells were bathed in extracellular solution containing 20 mM Ba^{2+}, and held at −60 mV. The Ba^{2+} current was activated by a 400 ms depolarizing step to +10 mV every 5 s. (a) The Ba^{2+} current in the absence and presence of 10 μM haloperidol recorded from a cell loaded with 2 mM GDPβS. An arrow indicates a zero current level. (b) Mean data. The amplitude of peak Ba^{2+} current in the presence of drugs was normalized to that just before the drug-application in individual cells, and the relative amplitude was compared between the cells loaded with standard extracellular solution containing 300 μM GTP (open columns) and those loaded with a solution containing 2 mM GDPβS (hatched columns). Data are mean ± s.e.mean from 4 to 5 cells. Asterisks indicate significant difference from control Ba^{2+} current determined by the paired *t* test using current amplitude before normalization.

from tricyclic antidepressants also produce a similar K^+ channel inhibition. In fact, five compounds that reduced the K^+ current in the present study (haloperidol, chlorpromazine, fluspirilene, SCH-23393 and W-7) differ in their structures from one another except that both haloperidol and fluspirilene are derivatives of phenylbutylpiperidine. In addition, within derivatives of phenylbutylpiperidine, pimozide did not significantly inhibit the K^+ current (Figure 4). Thus, ability to block the K^+ channels may not readily be estimated from simple molecular structures. Subclasses of voltage-gated Ca^{2+} channels are also blocked by various compounds related to haloperidol and chlorpromazine with a slight difference as to the potency of individual compounds. For example, haloperidol, chlorpromazine, fluspirilene and pimozide similarly suppressed ionic currents permeating through P-type Ca^{2+} channels in rat cerebellar Purkinje neurones and N-type Ca^{2+} channels in rat sympathetic neurones (Sah & Bean, 1994). These antipsychotic drugs also inhibited [^{3}H]-nitrendipine-binding, but the potency of fluspirilene and pimozide was about 100 fold higher than that of haloperidol or chlorpromazine in rat cerebral cortices (Gould *et al.*, 1983).

In spite of heterogeneity in structure, the block by haloperidol, chlorpromazine, SCH-23393 and W-7 of the K^+ current was similarly prevented, and the K^+ current block by fluspirilene was also attenuated by inclusion of GDPβS in the intracellular solution (Figure 5b). The result suggests that all of these compounds require GTP-binding proteins to inhibit the K^+ channels. The GTP-binding proteins underlying the K^+ channel inhibition do not seem to be PTX-sensitive (Figure 5d). The requirement of GTP-binding proteins can be interpreted into two different ways: 'mediation by GTP-binding proteins of the inhibition' and 'preliminary requirement of GTP-binding proteins for the inhibition'. The former idea is that these compounds activate GTP-binding proteins, which, in turn, lead the K^+ channels to a blocked state. The latter idea is that GTP-binding proteins are activated independent of these compounds, leading the K^+ channels to a specific state, and this state is selectively blocked by the compounds. Partial prevention by GTPγS of the K^+ current inhibition (Figure 5c) may support the former idea of 'mediation by GTP-binding proteins' if this prevention implies that preliminary activation by GTPγS of GTP-binding protein had partially inhibited the K^+ current before addition of haloperidol or chlorpromazine. However, this hypothesis is inconsistent with the lack of significant facilitation by GTPγS of the initial decline of the K^+ current at the beginning of current recordings. Another possibility that GDPβS and GTPγS prevent the K^+ current inhibition by acting on cellular components other than GTP-binding proteins cannot completely be excluded at present.

Modulations by neurotransmitters or other endogenous substances of ion channels through GTP-binding proteins have been well characterized, especially for voltage-gated Ca^{2+} channels and K^+ channels (see reviews, Yatani *et al.*, 1990; Hille, 1992; 1994). In cardiac cells, acetylcholine or adenosine activate K^+ channels through PTX-sensitive GTP-binding proteins (Kurachi *et al.*, 1986; Yatani *et al.*, 1990). On the other hand, acetylcholine and other endogenous substances reduce a K^+ current in peripheral neurones ('M current'; Brown, 1988), and extracellular ATP reduces a K^+ current in rat hippocampal neurones (Nakazawa *et al.*, 1994) through GTP-binding proteins. In addition, imipramine suppressed L-type Ca^{2+} channels in mouse dorsal root ganglia, and this suppression was prevented by PTX and attenuated by GDPβS (Choi *et al.*, 1992). It remains to be clarified whether the inhibition of the K^+ current by haloperidol or chlorpromazine shares common mechanisms with these modulations.

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