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Inhibition by antipsychotic drugs of L-type Ca^{2+} channel current in PC12 cells

Kanako Ito [a,b], Ken Nakazawa [a,*], Schuichi Koizumi [a], Min Liu [a], Kouichi Takeuchi [b], Takao Hashimoto [b], Yasuo Ohno [a], Kazuhide Inoue [a]

[a] *Division of Pharmacology, National Institute of Health Sciences, 1–18-1 Kamiyoga, Setagaya, Tokyo 158, Japan*
[b] *Department of Pharmacology, Meiji College of Pharmacy, 1–35-23 Nozawa, Setagaya, Tokyo 154, Japan*

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Abstract

Inhibition by antipsychotic drugs of voltage-gated L-type Ca^{2+} channels was characterized in rat neuronal cell line pheochromocytoma PC12 cells. Under whole-cell voltage-clamp, haloperidol and chlorpromazine (1–100 μM) inhibited Ba^{2+} current permeating through Ca^{2+} channels. Fluspirilene and pimozide inhibited the Ba^{2+} current at lower concentrations (fluspirilene, 0.1 pM to 1 nM; pimozide 10 pM to 1 μM). Effects of dopamine receptor antagonists and calmodulin antagonists were tested because antipsychotic drugs are known to exhibit these pharmacological activities. Sulpiride (1 and 10 μM), an antagonist to dopamine D_2 receptors, and SCH-23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; 1 and 10 μM), an antagonist to dopamine D_1 receptors, also inhibited the Ba^{2+} current. As for calmodulin antagonists, W-7 (*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; 10 and 100 μM) as well as calmidazolium (10 nM to 1 μM) reduced the Ba^{2+} current. The inhibition by haloperidol or fluspirilene of the Ba^{2+} current was not affected when GTP in intracellular solution was replaced with GDPβS. These properties of the Ca^{2+} channel inhibition are discussed by comparing with those of the K^+ channel inhibition and in relation to therapeutic relevance.

Keywords: Ca^{2+} channel; Antipsychotic drug; Dopamine receptor antagonist; Calmodulin antagonist; Membrane current

1. Introduction

Antipsychotic drugs exhibit the ability to relieve schizophrenic symptoms. Schizophrenic symptoms are divided into two classes, viz. positive symptoms, such as hallucinations and delusions, and negative symptoms, such as emotional withdrawal and poverty of speech. Most antipsychotic drugs, including haloperidol, relieve the positive symptoms, but less effective for the negative symptoms (Crown, 1980; Haas and Beckmann, 1982). One neuroleptic class, the diphenylbutylpiperidines, including fluspirilene and pimozide, are, however, also effective for the negative symptoms (Singh, 1973; Pinder et al., 1976; Frangos et al., 1978; Haas and Beckmann, 1982). The alleviation of positive symptoms has commonly been ascribed to antagonism at dopamine D_2 receptors because these two activities are well correlated (Seeman, 1980). In contrast, the antagonism at dopamine D_2 receptors does not correlate with the relief of the negative symptoms, and, thus, other mechanisms are necessary to explain this clinical activity.

Antipsychotic drugs have been reported to influence ion channels, including voltage-gated Ca^{2+} channels. The block of ionic current mediated through Ca^{2+} channels by fluspirilene, a well-known antipsychotic drug, was first demonstrated by Galizzi et al. (1986) in rabbit and rat skeletal muscle cells. In neuronal cells, Ogata et al. (1989) reported the inhibition by haloperidol of ionic current inhibition through Ca^{2+} channels in mouse neuroblastoma N1E-115 cells. More recent investigations have shown that haloperidol or other antipsychotic drugs inhibit various subclasses of the Ca^{2+} channels, including L-type Ca^{2+} channels in rat hippocampal neurons (Fletcher et al., 1994), T-type channels in rat pituitary growth hormone cell lines (Enyeart et al., 1990a), N-type channels in rat hippocampal (Fletcher et al., 1994) and rat sympathetic neurons (Sah and Bean, 1994), and P-type channels in rat cerebellar

* Corresponding author. Tel.: +81 3 3700 1141 (extension 348).

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Purkinje neurons (Sah and Bean, 1994). In addition, the effects of the new classes of drugs, including fluspirilene, on Ca^{2+} channels have recently been reviewed by Spedding et al. (1995) mainly in relation to their influence on cardiovascular systems.

The interaction of antipsychotic drugs with L-types Ca^{2+} channels was first demonstrated by Gould et al. (1983). They showed that antipsychotic drugs, including haloperidol and fluspirilene, inhibit [^{3}H]nitrendipine binding in membranes prepared from rat cerebral cortices. In addition to the inhibition by haloperidol in rat hippocampal neurons (Fletcher et al., 1994) as described above, the inhibition of ionic current mediated through these channels has also been reported for pimozide in rat cardiac cells (Enyeart et al., 1990b), and diphenylbutylpiperidine antipsychotic drugs, including fluspirilene and pimozide, in rat pituitary cell lines (Enyeart et al., 1990a). However, properties of the inhibition of these channels, such as relative potency of different classes of antipsychotic drugs or mechanisms underlying the channel inhibition have not been well clarified. Clarification of relative potency of different classes of antipsychotic drugs appears important because Antkiewicz-Michaluk et al. (1995) have recently pointed out the relation between withdrawal syndrome after treatment with these drugs and their potency to displace [^{3}H]nitrendipine binding. It is of interest to study whether such difference is observed, not only in the displacement of the binding of antagonists at L-type Ca^{2+} channels, but also in the function of these channels.

We previously characterized the inhibition by antipsychotic drugs of voltage-gated K^+ channels in rat pheochromocytoma PC12 cells, a peripheral neuronal cell line, and found that the inhibition involves a mechanism requiring GTP-binding proteins (Nakazawa et al., 1995). In undifferentiated PC12 cells, functional Ca^{2+} channels are mostly L-type (Plummer et al., 1989; Nakazawa et al., 1991), and N-type channels appear only when the cells are differentiated with nerve-growth factors (Plummer et al., 1989). Thus, undifferentiated PC12 cells are suitable to investigate influence on L-type Ca^{2+} channels. The present study was aimed at clarifying the properties of the inhibition by antipsychotic drugs of Ba^{2+} current permeating through these channels in PC12 cells.

2. Materials and methods

PC12 cells (passage 53–68) were cultured according to Inoue and Kenimer (1988). Cells were plated on collagen-coated coverslips placed on the bottom of 35-mm polystyrene dishes. After culturing for 1–3 days at 37°C, cells were used for experiments. When cells were treated with pertussis toxin, the toxin 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 (Inoue and Kenimer, 1988). 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 resistances of heat-polished patch pipettes ranged between 3 to 5 MΩ when the pipettes were filled with an intracellular solution containing (in mM) CsCl 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 CsOH). After the achievement of whole-cell recordings, the extracellular solution was switched to an extracellular solution containing 20 mM $BaCl_2$ ($CaCl_2$ and $MgCl_2$ were omitted, and the concentration of NaCl was reduced to 115 mM). Before application of drugs, Ba^{2+} current through voltage-gated Ca^{2+} channels was activated by a 400-ms depolarizing step to +10 mV from a holding potential of −60 mV every 5 s for about 3 min. At the end of each experiment, the Ca^{2+} channels were completely blocked by 30 μM Cd^{2+} (Nakazawa et al., 1991). The amplitude of the Ba^{2+} current was determined as difference from the current level with 30 μM Cd^{2+}. Experiments were performed at room temperature (about 25°C). Capacitance was compensated such that capacitative transient current upon voltage steps became minimal. 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 (Serenace 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-1*H*-3-benzazepine; Research Biochemicals, Natick, MA, USA), (−)-sulpiride (Research Biochemicals), W-7 (*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; Sigma, St. Louis, MO, USA), calmidazolium (compound R24571; Sigma), ATP (adenosine 5'-triphosphate disodium salt; Sigma), GTP (guanosine triphosphate sodium salt; Sigma), and GDPβS (guanosine 5'-*O*-(2-thiodiphosphate) trilithium salt; Sigma). Pertussis toxin was a kind gift of Dr. J.G. Kenimer. Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan). Pimozide, fluspirilene, SCH-23390, sulpiride, W-7 and calmidazolium were first dissolved in dimethylsulphoxide (DMSO), and then diluted with extra- or intracellular solutions so that final concentration of DMSO was less than 1%. In preliminary experiments, 1% DMSO did not affect the 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 report were given as mean ± S.E.M. Statistical difference was determined at the level of 0.05 ($P < 0.05$) using Student's *t*-test.

3. Results

3.1. Effects of antipsychotic drugs on Ba^{2+} current

When PC12 cells were bathed in an extracellular solution containing 20 mM Ba^{2+} as the only divalent cation and a voltage step to +10 mV was applied from a holding potential of −60 mV, an inward current was activated (Fig. 1A). Previous reports have demonstrated that the inward current is Ba^{2+} current permeating through L-type Ca^{2+} channels (Plummer et al., 1989; Nakazawa et al., 1991). We also confirmed in preliminary studies that the Ba^{2+} current is sensitive to 1 μM nicardipine (not shown). The Ba^{2+} current was inhibited by haloperidol (1 and 10 μM; Fig. 1A). The inhibition was observed immediately after the beginning of the application of haloperidol, progressively increased and reach to steady-state within 30 s. Upon washout, the Ba^{2+} was readily recovered and reached

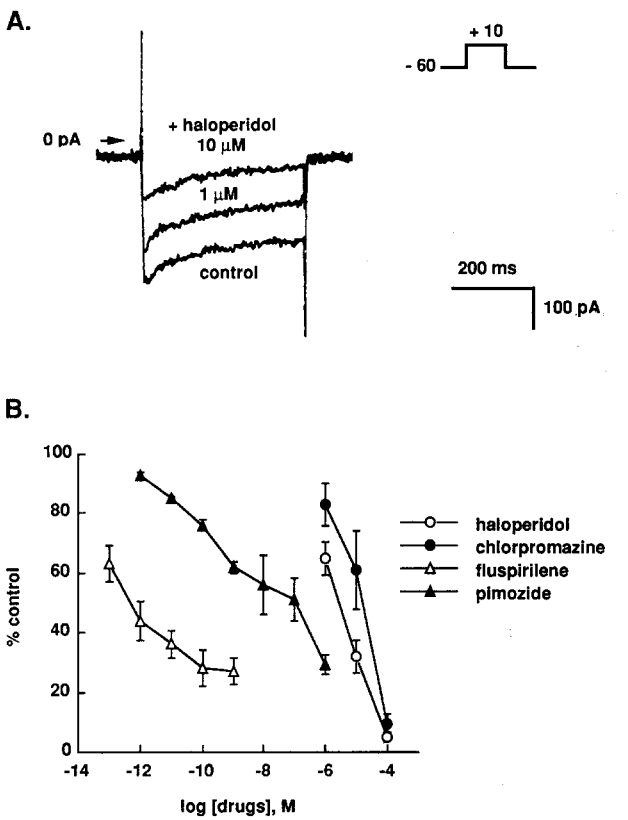

Fig. 1. Inhibition by haloperidol and other antipsychotic drugs of Ba^{2+} current permeating through L-type Ca^{2+} channels in PC12 cells. Cells were held at −60 mV, and a 400-ms depolarizing step to +10 mV was applied every 5 s. During repetitive depolarization, drugs were administered for 1 min. (A) Ba^{2+} currents activated by the voltage step in the absence and presence of 1 and 10 μM haloperidol. An arrow indicates a zero current level. (B) Concentration-response relationship for the Ba^{2+} current inhibition by haloperidol (open circles), chlorpromazine (filled circles), fluspirilene (open triangles) and pimozide (filled triangles). Peak Ba^{2+} current with the drugs was normalized to that just before the drug application in individual cells. Data were mean ± S.E.M. obtained from 4–8 cells.

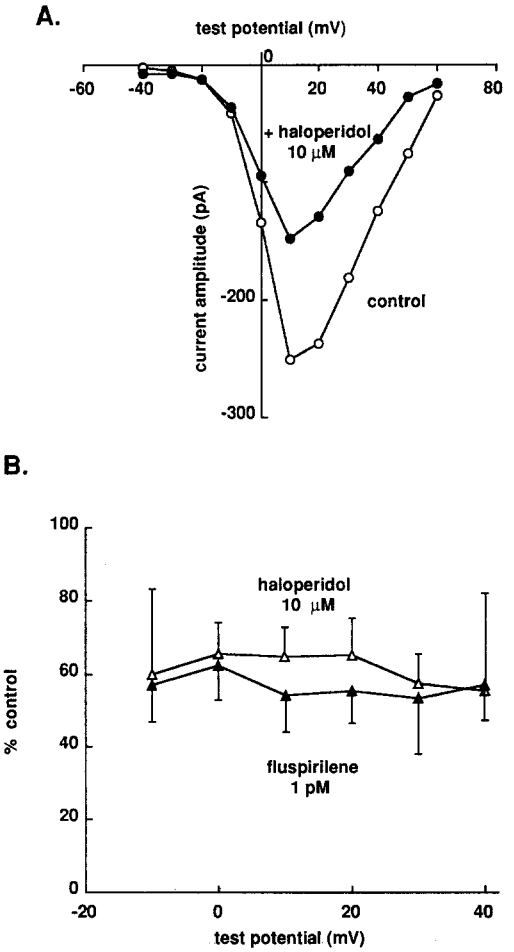

Fig. 2. Inhibition by haloperidol (10 μM) and fluspirilene (1 pM) of Ba^{2+} current at various test potentials. Cells were held at −60 mV and 400-ms voltage steps in a 10-mV increment were applied every 5 s. (A) Current-voltage relationship for peak Ba^{2+} current in the absence (open circles) and presence of haloperidol (filled circles). Current amplitude was plotted against test potentials. (B) Current fraction remaining after block by haloperidol (open triangles) or fluspirilene (filled triangles) at various test potentials. Peak Ba^{2+} current with drugs were normalized to that before the drug application at each test potential in individual cells. Data were mean ± S.E.M. from 4 cells.

the control level within a 1 min rinse with drug-free solution. The concentration-response relationship of haloperidol was obtained with cumulative application (Fig. 1B). From the concentration-response relation, EC_{50} was roughly estimated to be 20 μM (Fig. 1B). Effects of other antipsychotic drugs were examined. Chlorpromazine also reversibly inhibited the Ba^{2+} current in a similar concentration range (EC_{50} was about 3 μM; Fig. 1B). Fluspirilene inhibited the Ba^{2+} current in a lower concentration range; the inhibition was observed at concentrations as low as 0.1 pM, and EC_{50} was about 1 pM (Fig. 1B). The inhibition by fluspirilene appeared to be irreversible: no recovery of the current was observed after a rinse up to 15 min. Pimozide also inhibited the Ba^{2+} current: it blocked the current and EC_{50} was about 100 nM (Fig. 1B). Like fluspirilene, the inhibition by pimozide was also irreversible.

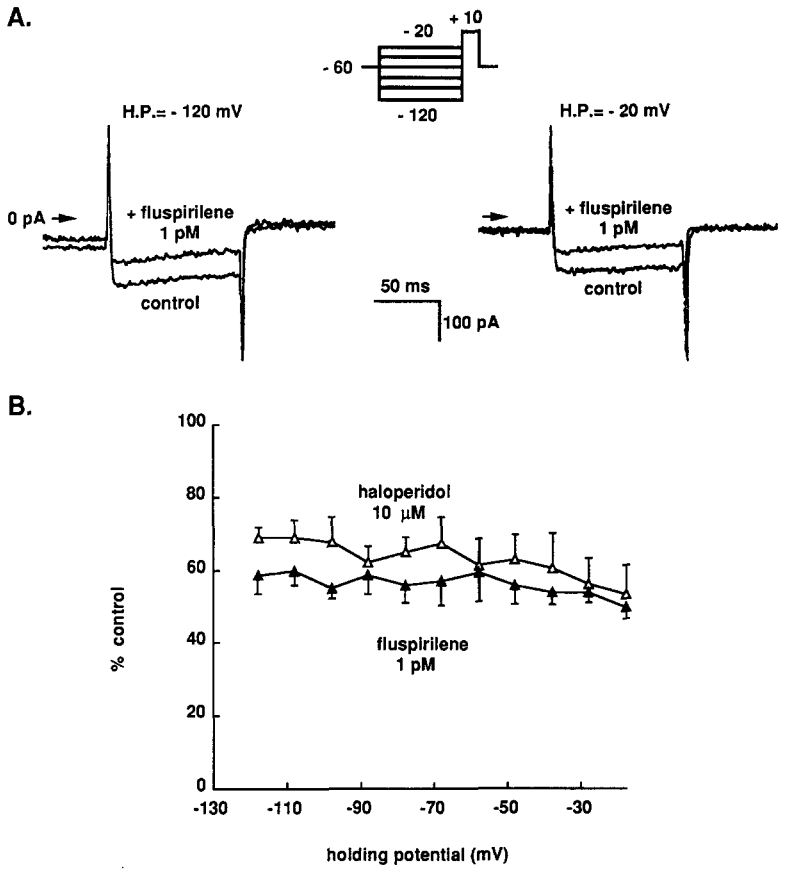

Fig. 3. Inhibition by haloperidol (10 μM) and fluspirilene (1 pM) of Ba^{2+} current activated from various holding potentials. Before the Ba^{2+} current was activated by a 200-ms depolarizing step to +10 mV, cells were held at various potentials by applying a 300-ms prepulse from −60 mV. (A) Inhibition by 1 pM fluspirilene of Ba^{2+} current activated from −120 (left) and −20 mV (right). Current traces with and without fluspirilene were superimposed in each panel. Arrows indicate zero current levels. Note that the holding current at −120 mV was more inward with fluspirilene compared with the control current in the left panel (see also text). (B) Current fraction remaining after block by haloperidol (open triangles) or fluspirilene (filled triangles) obtained with various holding potentials. The peak Ba^{2+} current with drugs were normalized to that before the drug application in individual cells. Data were mean ± S.E.M. from 4 cells.

In addition to the difference in the potency to inhibit the Ba^{2+} current, other aspects of the inhibition were diverse among these drugs. With fluspirilene or pimozide, the slope of the concentration-response curve was less steeper than that with haloperidol or chlorpromazine, and they did not totally block the current at the highest concentration tested (Fig. 1B).

3.2. Dependence of Ba^{2+} current inhibition on membrane potentials

Fig. 2A shows current-voltage (I-V) relationship for the Ba^{2+} current obtained with various depolarizing test potentials applied from a holding potential of −60 mV. The I-V relationship exhibited a bell-shape peaking at +10 mV, as has been reported (Nakazawa et al., 1991). Haloperidol (10 μM) blocked the Ba^{2+} current at all the test potentials, and the I-V relation did not appear to be affected. To accentuate the lack of influence on the I-V relationship, we plotted the current component remaining after the current inhibition against the test potentials in Fig. 2B. The results suggest that the inhibition by haloperidol or fluspirilene (1 pM) does not depend on potentials for the Ba^{2+} current activation.

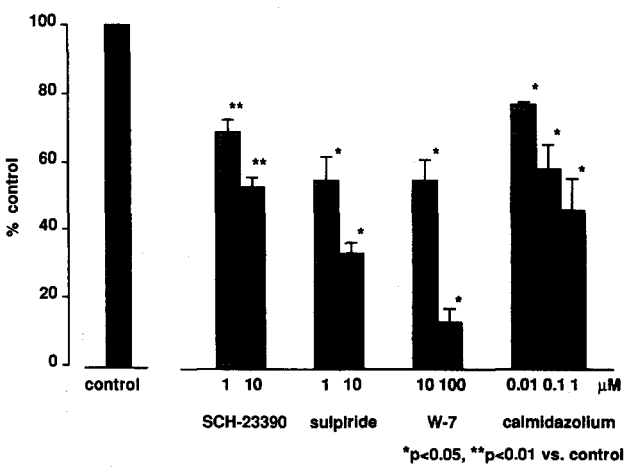

Fig. 4. Effects of dopamine receptor antagonists and calmodulin antagonists on Ba^{2+} current. Data were obtained and shown as Fig. 1. Each column and bar represent mean ± S.E.M. from 4–6 cells.

To examine dependence on holding potentials of the Ba^{2+} current inhibition, 300-ms prepulses of −120 to −20 mV was applied before activation of the Ba^{2+} current at +10 mV (Fig. 3A). The amplitude of the Ba^{2+} current was only slightly decreased with a more depolarized prepulse, suggesting that Ca^{2+} channels responsible for the Ba^{2+} current exhibit weak voltage-dependent inactivation, as commonly observed for L-type Ca^{2+} channels (e.g. Hess, 1990). Haloperidol (10 μM) or fluspirilene (1 pM) reduced the Ba^{2+} current activated from all the prepulses tested. Fig. 3B illustrates the current component remaining after the inhibition plotted against the prepulses. Although both the magnitude of the current inhibition by haloperidol and that by fluspirilene appeared to be slightly potentiated with a more depolarized prepulse, the potentiation was not statistically significant (Student's *t*-test, $P > 0.05$).

The holding current was more inward with fluspirilene than the control current at −120 mV, but not at −20 mV. A similar inward shift was also observed with haloperidol, as previously reported (Nakazawa et al., 1995). We have not clarified the properties of this small inward current

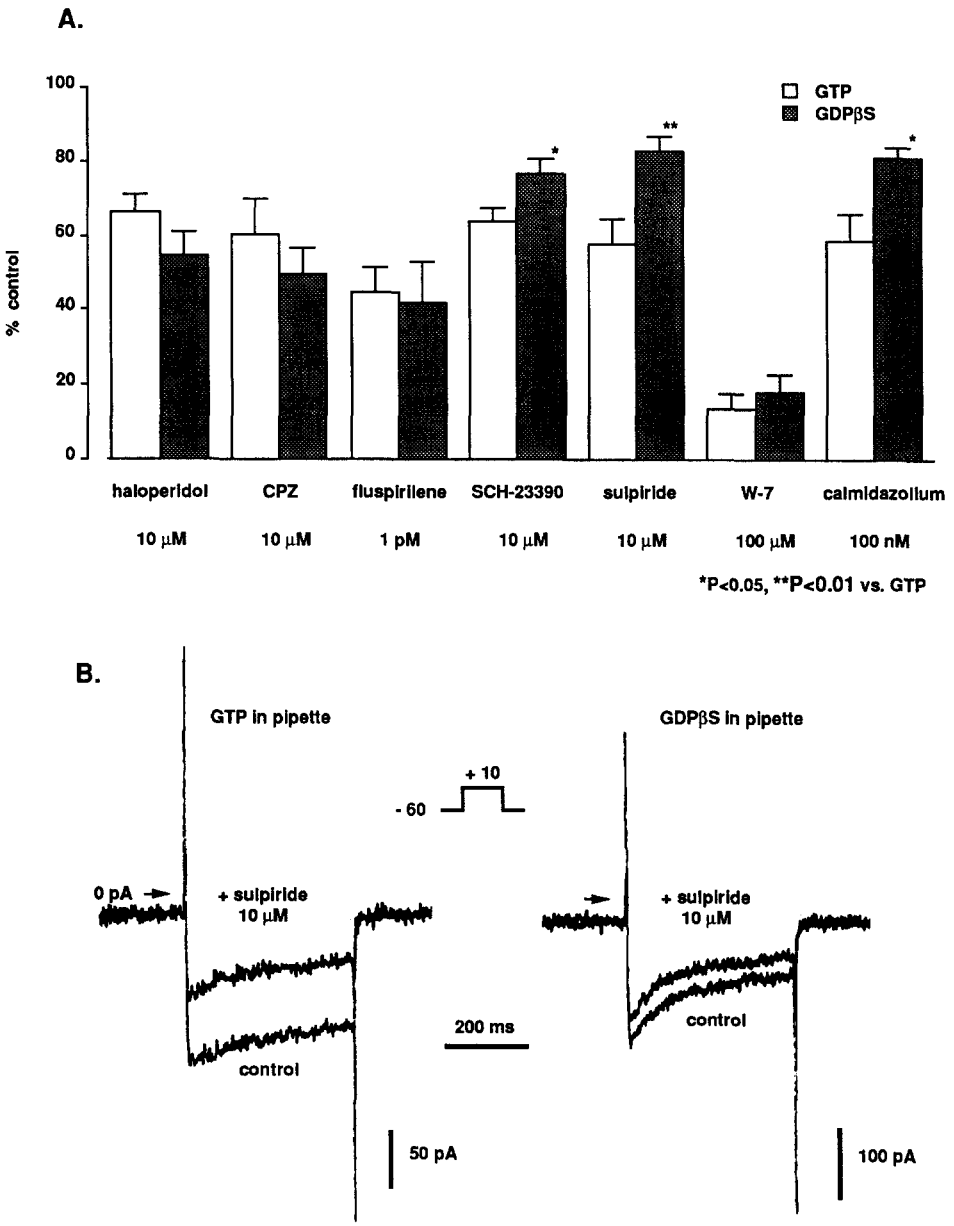

Fig. 5. (A) Influence of GDPβS on the Ba^{2+} current inhibition. The current remaining after the inhibition with standard intracellular solution (open columns) and that with intracellular solution containing GDPβS (hatched columns) were compared for each compound. Data were obtained from 4–9 cells tested and shown as in Fig. 1. Asterisks indicate significant difference from the inhibition with the standard solution determined by Student's *t*-test (* $P < 0.05$). (B) Ba^{2+} current in the absence and presence of 10 μM sulpiride in a cell loaded with a standard intracellular solution (left) or another cell loaded with an intracellular solution containing 2 mM GDPβS instead of GTP. Arrows indicate zero current levels.

induced by these compounds. However, this inward current cannot produce significant errors in estimating the amplitude of the Ba^{2+} current, because the inward current was negligible at positive potentials.

3.3. Effects of related compounds

The effects of compounds pharmacologically related to antipsychotic drugs were tested (Fig. 4). Antipsychotic drugs generally antagonize dopamine D_2 receptors (Seeman, 1980). Sulpiride, a selective antagonist of dopamine D_2 receptors, as well as SCH-23390, a selective antagonist of dopamine D_1 receptors inhibited the Ba^{2+} current at 1 and 10 μM. Haloperidol and chlorpromazine antagonize calmodulin (Levin and Weiss, 1979; Hidaka et al., 1980). Two well-known calmodulin antagonists, W-7 and calmidazolium also inhibited the Ba^{2+} current at concentrations that inhibit calmodulin-dependent responses (W-7, 10 and 100 μM; Hidaka et al., 1980: calmidazolium, up to 1 μM; Gietzen et al., 1981).

3.4. Contribution of GTP-binding proteins

Antipsychotic drugs and related compounds inhibit a voltage-activated K^+ current in PC12 cells, and addition of GDPβS, an inhibitor of GTP-binding proteins, in intracellular solution attenuates the K^+ current inhibition (Nakazawa et al., 1995). A similar test was made to determine whether GTP-binding proteins also contribute to the Ba^{2+} current inhibition (Fig. 5A). Unlike the K^+ current inhibition, the Ba^{2+} current inhibition by haloperidol was not attenuated when GTP in intracellular solution was replaced with 2 mM GDPβS. The replacement with GDPβS also did not affect the Ba^{2+} current inhibition by chlorpromazine and fluspirilene. In contrast, GDPβS significantly attenuated the Ba^{2+} current inhibition by SCH-23390 and that by sulpiride (Fig. 5B). As for calmodulin antagonists, GDPβS significantly attenuated the inhibition by W-7, but it did not affect that by calmidazolium. The inhibition by SCH-23390 or sulpiride was also attenuated by the treatment of cells with pertussis toxin. In pertussis toxin-treated cells, the magnitude of the Ba^{2+} current inhibition was significantly reduced from 36.2 ± 3.6 to $22.7 \pm 2.7\%$ (SCH-23390 10 μM) or from 42.1 ± 6.7 to $16.8 \pm 1.5\%$ (sulpiride 10 μM), respectively (Student's t-test, $P < 0.05$).

4. Discussion

We have investigated properties of inhibition by antipsychotic drugs on Ba^{2+} current permeating through voltage-gated L-type Ca^{2+} channels in PC12 cells. We found that the Ba^{2+} current is more sensitive to fluspirilene than haloperidol and chlorpromazine, and that the inhibition by haloperidol or fluspirilene of the Ba^{2+} current, unlike that of the K^+ current, does not require GTP-binding proteins.

All the antipsychotic drugs tested in this report inhibited the Ba^{2+} current permeating through L-type Ca^{2+} channels in PC12 cells, but fluspirilene and pimozide are more potent than haloperidol or chlorpromazine (Fig. 1B). In accord with these results, Gould et al. (1983) reported that fluspirilene and pimozide inhibit [^{3}H]nitrendipine binding at nanomolar concentrations whereas haloperidol and chlorpromazine inhibit it at micromolar concentrations. Galizzi et al. (1986) also reported that fluspirilene blocks ionic currents through L-type Ca^{2+} channels in rabbit skeletal muscle cells at nanomolar concentrations. In contrast, the block by fluspirilene or pimozide of P-type or N-type Ca^{2+} channels required concentrations of several tens of nanomolars or micromolars (Grantham et al., 1994; Sah and Bean, 1994). Thus, the blockade by fluspirilene and pimozide at nanomolar or subnanomolar concentrations may be peculiar to L-type Ca^{2+} channels.

Dopamine receptor antagonist and calmodulin antagonists also inhibited the Ba^{2+} current (Fig. 4). Antipsychotic drugs are known to antagonize dopamine D_2 receptors (Seeman, 1980) or calmodulin (e.g. Levin and Weiss, 1979). However, these antagonistic activities may not be related to the Ca^{2+} channel inhibition, judging from difference in susceptibility to GDPβS (Fig. 5): GDPβS did not affect the Ba^{2+} current inhibition by haloperidol, chlorpromazine or fluspirilene though it attenuated the current inhibition by sulpiride, a dopamine D_2 receptor antagonist, or that by calmidazolium, a calmodulin antagonist. These results suggest that sulpiride or calmidazolium inhibit L-type Ca^{2+} channels through a mechanism involving GTP-binding proteins whereas the inhibition by haloperidol, chlorpromazine or fluspirilene does not involve this mechanism. The contribution of GTP-binding proteins was also reported for the inhibition by imipramine of L-type Ca^{2+} channels in mouse dorsal root ganglion neurons (Choi et al., 1992).

The Ba^{2+} current inhibition in PC12 cells is similar to the inhibition of the voltage-activated K^+ current in these cells (Nakazawa et al., 1995) in the points that haloperidol and chlorpromazine inhibit the currents at micromolar concentrations, and that a variety of pharmacologically related compounds (dopamine receptor antagonists and calmodulin antagonists) also mimic the current inhibition. However, the following points are different between these two types of current inhibition: (1) the K^+ current inhibition by antipsychotic drugs, including haloperidol and fluspirilene, requires GTP-binding proteins, but the Ba^{2+} current inhibition by these compounds dose not; and (2) the Ba^{2+} current is more sensitive to fluspirilene and pimozide than to haloperidol and chlorpromazine whereas the K^+ current exhibited similar sensitivities to these compounds.

In contrast to limited clinical actions of haloperidol and chlorpromazine on positive symptoms of schizophrenia

(Crown, 1980), fluspirilene and pimozide are effective to negative symptoms as well as positive symptoms (Singh, 1973; Pinder et al., 1976; Frangos et al., 1978; Haas and Beckmann, 1982). Thus, judging from higher sensitivities of L-type Ca^{2+} channels to fluspirilene and pimozide (Fig. 1B), the inhibition of these channels may be related to such different spectra of clinical actions. This possibility has also been speculated on by Gould et al. (1983) on the grounds of the different potencies for the inhibition of [^{3}H]nitrendipine binding. As the concentrations of fluspirilene and pimozide required for the L-type channel inhibition (Fig. 1B) were comparable to or even smaller than those displaced [^{3}H]spiperon labeling dopamine D_2 receptors (Gould et al., 1983), it is highly likely that this channel inhibition occurs at clinically available doses. In addition to clinical actions, the difference in the potency of the current inhibition in the present study may be related to withdrawal syndrome after treatment with neuroleptics, as pointed out by Antkiewicz-Michaluk et al. (1995). They have shown that treatment with haloperidol, but not with pimozide, produces withdrawal symptoms, such as increase in spontaneous motor activity, and that pimozide displaced [^{3}H]nitrendipine binding at nanomolar concentrations whereas haloperidol displaced it at micromolar concentrations. Our present finding that pimozide was more potent than haloperidol in blocking the Ba^{2+} current supports their speculation that the lack of induction by pimozide of the withdrawal symptom arises from strong inhibition by this compound of the activity of L-type Ca^{2+} channels. The irreversible nature of the current inhibition by pimozide, in contrast to reversible inhibition by haloperidol, may also related to the lack of the induction of the withdrawal symptom. Furthermore, as L-type Ca^{2+} channels are widely distributed (Bean, 1989; Hess, 1990), the inhibition by nanomolar concentrations of fluspirilene and pimozide of L-type Ca^{2+} channels may produce unknown side-effects when they act on peripheral neurons or non-neuronal cells.

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