Short communication

Effects of Haloperidol and Chlorpromazine on Smooth Muscle Contractility, Platelet Aggregation and Neuronal Calcium Current

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Abstract. Effects of chlorpromazine, haloperidol (neuroleptics and calmodulin antagonists), and verapamil on rat platelet aggregation induced by thrombin, on calcium current in snail neurones and on both tonic tension of high potassium contracture and phasic contraction of isolated guinea-pig ureter preparations were studied. Moreover, droperidol, sulpiride and prazosine effects were studied for models of phasic contractility and platelet aggregation. Sulpiride and prazosine were ineffective, verapamil was ineffective on platelet aggregation, while droperidol was the most potent inhibitor of platelet aggregation. These results, the similarity revealed in the blockage of neuronal calcium current by neuroleptics and verapamil, and the potent inhibitory action of haloperidol and chlorpromazine on contractility and aggregation suggest that both phenothiazine and butyrophenone neuroleptics possess some properties of calcium antagonists and may also have intracellular sites of action other than calmodulin.

Key words: Neuroleptics — Verapamil — Calcium channels — Smooth muscle contractility — Platelet aggregation

Chlorpromazine and other phenothiazines, neuroleptics and calmodulin antagonists, are known to inhibit some Ca^{2+}-dependent processes such as muscle contraction (Tetsuyuki 1989; Eto et al. 1991) and platelet aggregation (Smith et al. 1990). Another calmodulin antagonist, a butyrophenone, the antipsychotic agent haloperidol and some other butyrophenones have also been shown to inhibit these processes (Chang et al. 1987; Seth et al. 1991; Hishinuma et al. 1992; Bertha et al. 1993). Along with calmodulin inhibition, neuroleptic ability to block Ca^{2+} channels (Mannhold 1988; Enyeart et al. 1992) can be of great importance for calcium antagonistic action of neuroleptics. Due to the fundamental role of Ca^{2+} in

organism, the study of calcium antagonist properties of neuroleptics may promote our understanding of some mechanisms of their side effects and a more effective using of these drugs in clinical pharmacology. A certain success of attempts to apply verapamil to treatment of schizophrenia and mania (Raeburn and Gonzola 1988; Baghi 1990) makes the investigation of neuroleptic effects on Ca^{2+}-dependent cellular processes in comparison with those of verapamil particularly interesting.

In the present study the actions of chlorpromazine and haloperidol, the typical representatives of the two major neuroleptic groups (phenothiazine and butyrophenone derivatives), and calmodulin antagonists, on smooth muscle contractility (both phasic contraction evoked by electrical field stimulation and tonic tension of high potassium contracture), platelet aggregation and neuronal calcium current were studied and compared with those of the classic calcium antagonist, verapamil. Since the specific pharmacological targets of both phenothiazine and butyrophenone neuroleptics are surface receptors of central neurons, especially D_2-dopamine receptors and α_1-adrenoreceptors (LaBella 1991), the influence of the specific antagonists of both D_2-dopamine receptors (sulpiride, benzamide neuroleptic) and α_1-adrenoceptors (prazosine), as well as of another butyrophenone antipsychotic agent, droperidol, were also studied using experimental models of phasic contractility and platelet aggregation.

The potential-gated calcium current was investigated on voltage-clamped, non-identified isolated neurons of the snail *Helix pomatia* by the method of intracellular perfusion (Kostyuk et al. 1981). The extracellular solution to register calcium currents was of the following composition (in mmol/l): $CaCl_2$ 20; $MgCl_2$ 4; Tris-HCl 92; pH 7.55. The intracellular solution contained CsCl 50; $MgCl_2$ 3; EGTA-Na_2 1; ATP-Na_2 2; Tris-aspartate 70; pH 7.5. All experiments were performed at room temperature (20–22 °C).

Blood was collected from the hearts of adult male Wistar rats anesthetized with ether. Platelet-rich plasma was prepared from citrated fresh blood by centrifugation at $200 \times g$ for 20 min. The concentration of cells was brought to $1.5 \cdot 10^5$ platelets per μl, being checked by the optical density of suspension. The aggregation of a stirred platelet suspension at 37 °C was studied in an aggregometer by the method of Cazenave et al. (1983), measuring the initial aggregation rate. The suspension volume was 2 ml. Cell suspension was preincubated for 2 min, then 20 μl of the studied drug solution was added (final concentrations 1–30 μmol/l). One minute later, 20 μl of thrombin solution was added to a final concentration of 0.25 U/ml.

The contractile responses were recorded isometrically using the continuous superfusion technique described in detail by Brading and Sneddon (1980). The experiments were carried out with isolated guinea-pig ureter preparations, about 12 mm in length, taken from the middle part of the organ, from males weighing 400–600 g. The modified Krebs solution used was of the following composition

(in mmol/l): NaCl 120.3; KCl 5.9; $CaCl_2$ 2.5; $MgCl_2$ 1.2; glucose 11.5; and Tris-HCl 16.6; pH 7.4 at 37°C (bubbled with 100% O_2). Phasic contractions were evoked by right-angled current pulses (about 10^{-7} A) of short duration ($\leq$ 0.1 s) via silver chloride electrodes. High potassium depolarization was provided by addition to Krebs solution of 100 mmol/l KCl as a correspondent amount of dry salt. Drugs used in this study were verapamil, chlorpromazine, droperidol, sulpiride, prazosine (Sigma Chemical Co., St. Louis, Mo.) and ampoule solutions of 5 mg/ml haloperidol (Gedeon Richter, Budapest, Hungary). The drug concentrations were no more than 30 μmol/l for sulpiride and prazosine, and 100 μmol/l for the other drugs, except 150 μmol/l for haloperidol in model of neuronal calcium current, thus excluding a possibility of solvent effect in the models used.

The data are expressed as means ± S.E.M from 4–6 separate experiments. Comparison of mean values was performed using unpaired Student's t-test. Differences were considered significant when $P < 0.05$. The 50% inhibitory concentration

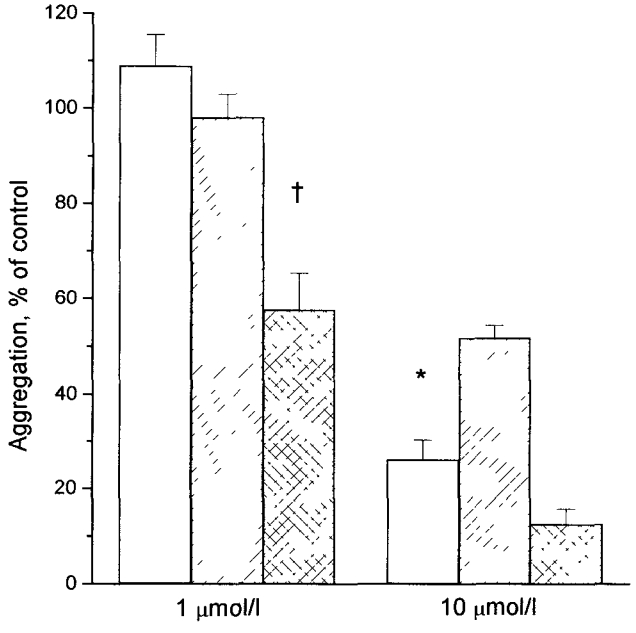

Figure 1. Effect of chlorpromazine (open bar), haloperidol (hatched bar) and droperidol (cross-hatched bar), in concentrations of 1 μmol/l and 10 μmol/l, on the initial rate of rat platelet aggregation induced by thrombin.
† $P < 0.005$ vs. haloperidol or chlorpromazine;
* $P < 0.05$ vs. droperidol or haloperidol.
Note: Verapamil, sulpyride and prazosine were ineffective in concentrations up to 30 μmol/l.

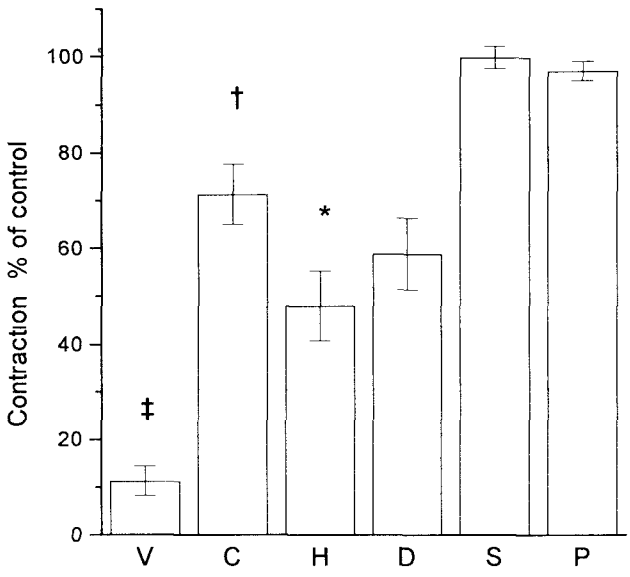

Figure 2. Effect of studied drugs (30 μmol/l) on phasic contraction produced by electrical field stimulation of isolated guinea-pig ureter preparations. V verapamil, C chlorpromazine, H haloperidol, D droperidol, S sulpyride and P prazosine.
‡ $p < 0.005$ vs haloperidol
* $P < 0.05$ vs chlorpromazine
† $P < 0.01$ vs sulpyride or prazosine

(IC_{50}) values were calculated from the concentration response curves of the inhibition of contractile response, platelet aggregation (initial rate) and calcium current maximum amplitude by chlorpromazine, haloperidol and verapamil.

Both chlorpromazine (CPZ) and haloperidol (HLP) had dose dependent inhibitory effects in all the models used. Table 1 shows the respective IC_{50} values in comparison with those found for verapamil. As one can see from the Table as well as from Figs. 1 and 2, CPZ preferentially inhibited neuronal calcium current and platelet aggregation, while HLP preferentially inhibited phasic contraction evoked by electrical field stimulation. For the models of phasic contractility and platelet aggregation the specific antagonists of D_2 dopamine receptors (sulpiride) and α_1 adrenoceptors (prazosine) in concentrations up to 30 μmol/l had no effect (Fig. 2), suggesting that pharmacofores or parts of neuroleptic molecules, which provide specificity interaction with the corresponding receptors, may not be involved in the neuroleptic effects.

Droperidol acted like HLP did on phasic contraction (Fig. 2) and was con-

Table 1. Comparison of the inhibitory effects (IC_{50}) of neuroleptics and verapamil on both phasic contraction (evoked by electrical field stimulation) and tonic tension of high potassium (106 mmol/l) contracture of isolated guinea-pig ureter preparations, on the initial rate of rat platelet aggregation induced by thrombin and on the maximum amplitude of calcium current through high threshold L-type-like channels of isolated snail neurons

Drug	IC_{50}, μmol/l			
	Calcium current	Phasic contraction	Tonic tension	Platelet aggregation
Haloperidol	135 ± 19*	28.3 ± 3.6*	3.5 ± 0.6	9.5 ± 1.8*
Chlorpromazine	62 ± 12	45.6 ± 6.9	5.0 ± 1.1	4.2 ± 0.9
Verapamil	74 ± 15	10.9 ± 3.0**	0.17 ± 0.04**	*a*

Note: Values are means ± S.E.M ($n = 4 \div 6$ animals). Comparison between means were performed using unpaired Student's *t*-test.
a ineffective up to 30 μmol/l of drug.
* $P < 0.05$ vs. chlorpromazine;
** $P < 0.01$ vs. haloperidol or chlorpromazine.

siderably more potent as platelet aggregation inhibitor than both HLP and CPZ (Fig. 1). Inhibitory effect of droperidol on aggregation of porcine platelets and Ca^{2+} mobilization in human platelets was also previously reported by Bertha et al. (1993). Verapamil, in contrast to neuroleptics, did not show significant effect (in concentrations up to 30 μmol/l) on thrombin-induced aggregation of rat platelets; this is in good agreement with the data on the CPZ and verapamil inhibition of ADP-induced primary aggregation of human platelets (Smith et al. 1990). In the reffered work triphtazine, an other phenothiazine and calmodulin antagonist, similar to verapamil, was ineffective; thus, the antiaggregation effect of these neuroleptics cannot be explained by either their interaction with calmodulin or inhibition of the platelet sarcolemma Ca^{2+} channels. In our case, the potent anti-aggregation effect of droperidol allows to propose that also in rat platelets the neuroleptics have intracellular sites of action other than calmodulin.

In experiments with non-identified isolated neurons from *Helix pomatia*, both CLP and HLP, similarly to verapamil, reversibly blocked current through high threshold L-type-like calcium channels, when applied externally (Table 1). Current inhibition usually proceeds in two phases: the initial quick phase which lasts 20–40 s, and the slow phase which lasts 3–5 min. The current recovers in 2–6 min after drug washout. When applied intracellularly, drugs at 50 μmol/l had no effect on calcium current. This observation is similar to that of effective extracellular application of calcium antagonists in neurons from *Helix pomatia* (Nishi et al.

1983). Similarly to verapamil and CPZ, HLP seems to block neural Ca^{2+} channels directly. Ca^{2+} channel blocking may play an important role in inhibition of muscle contraction by CPZ (Eto et al. 1991) and HLP (Seth et al. 1991).

As one can see from the Table 1, similarly to nifedipine and diltiazem (Burdyga and Magura 1987) verapamil blocks the tonic component of high potassium contracture, reflecting the effect on voltage-operated Ca^{2+} channels opened during sustained depolarization (Burdyga and Magura 1987), approximately 50–100 times more effectively than they act on the phasic contraction associated with the acting potential. Though in the used models of smooth muscle contractility, especially tonic, neuroleptics were less potent than verapamil, their potency can be compared with another known calcium antagonist as well as phosphodiesterase (PDE) and platelet aggregation inhibitor, papaverine (Brading et al. 1983). The inhibitory effect of the latter on L-type Ca^{2+} channel activity in smooth muscle cells was shown recently (Liem et al. 1994). PDE may be one of the common non-specific targets which are inhibited by CPZ and HLP at high concentrations used in the model of phasic contractility (LaBella 1991). However these concentrations exceed IC_{50} values of the inhibitory effects of the neuroleptics for this model. CPZ and other neuroleptics, calmodulin antagonists, are known to suppress Ca^{2+} activated contraction of both blood vessel and intestinal skinned smooth muscles, practically at the same concentrations these drugs cause intact smooth muscle relaxation (Tetsuyuki 1989). CPZ is a more potent Ca^{2+} channel blocker and calmoduline antagonist than haloperidol. Besides, CPZ but not HLP is also protein kinase C (PKC) inhibitor (Kodavanti and Mehendale 1990; LaBella 1991). Since the inhibitory potency of HLP is not less than that of CPZ on isolated ureter it may be suggested that in addition to calmodulin and PKC, the neuroleptics might also have other intracellular targets in the guinea-pig ureter smooth muscle.

CPZ is a cationic amphiphilic drug (CAD) known to be a potent inhibitor of phospholipases (PLes) (Kodavanti and Mehendale 1990). Chloroquine, another CAD and a potent inhibitor of PLA_2, dose-dependently inhibited thrombin-induced aggregation of human and rat platelets as well as arachidonic acid liberation from membrane phospholipids, malondialdehyde formation and thromboxane production (Nosáľ et al. 1994; Nosáľ et al. 1995). Ca^{2+}-regulated PLA_2 and platelet aggregation may also be potently inhibited by some butyrophenone derivatives (Chang et al. 1987). Besides, in thrombin stimulated human platelets, CPZ depressed phosphatidylinositol (PI) 4,5-bisphosphate (P_2) metabolism and inositol 1,4,5-triphosphate formation, probably by means of PLC inhibition (Pandey et al. 1991; Tsukahara et al. 1991); both CPZ and HLP inhibited phosphorylation of the 40 kDa protein by PKC, as well as phosphatidic acid formation (Tsukahara et al. 1991), and increased accumulation of PI, PI-4-phosphate and PIP_2 (Pandey et al. 1991). The potencies of the individual neuroleptics in the experimental models used, with the exception of the model of neural calcium current, cannot be rea-

sonably explained by the interactions with calmodulin, Ca^{2+} channels, PKC and PDE, and suggest that important components of the Ca^{2+}-mobilizing phospholipid-signalling system such as PLes C and/or A_2, phosphoinositide and/or arachidonic acid cascades (Kodavanti and Mehendale 1990; Nosál et al. 1995) may be single or combined principal targets of phenothiazine and butyrophenone neuroleptics in rat platelets and guinea-pig ureter smooth muscle. Probably, the non-specificity of CPZ, HLP and other antagonist drugs (LaBella 1991; Nosál et al. 1995), particularly of those able to inhibit the phospholipid-signalling system (Poda and Tanchuk 1994; Prokopenko et. al. 1994; Prokopenko et. al. 1995), is a result of commonalities revealed in the chemical structures of these drugs (LaBella 1991; Poda and Tanchuk 1994; Prokopenko et. al. 1995) and functional proteins (LaBella 1991).

Thus, the results obtained from four experimental models used indicate that both CPZ and HLP are effective inhibitors of different Ca^{2+}-dependent processes, showing properties similar to those of calcium antagonists such as verapamil and papaverine, and suggest that in addition to calmodulin, neuroleptics have sites of action in rat platelets and guinea-pig ureter, determining their inhibitory effects on aggregation and contraction. This inhibition may result, at least in part, from direct or indirect depressant actions of the neuroleptics on one or more important components of the phospholipid-signalling pathway.

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References

Baghi S. P. (1990): Antidopaminergic action of verapamil and several other drugs: inactivation of vesicular dopamine. Life Sci. **46,** 857—863

Bertha B. G., Sill J. G., Berger J., Folts J. O., Milde J. H. (1993): High-dose droperidol protects against coronary thrombosis in dogs and pigs and attenuates aggregation of porcine platelets and Ca^{2+} mobilization in human platelets. Anesthesiology **78,** 733—743

Brading A. F., Sneddon P. (1980): Evidence for multiple sources of calcium for activation of the contractile mechanisms of guinea-pig taenia coli on stimulation with carbachol. Brit. J. Pharmacol. **70,** 229—240

Brading A. F., Burdyga Th. V., Scripnyuk Z. D. (1983): The effects of papaverine on the electrical and mechanical activity of the guinea-pig ureter. J. Physiol. (London), **334,** 79—89

Burdyga Th. V., Magura I. S. (1987): Effects of diltiazem on the electrical and mechanical activity of the guinea-pig ureter smooth muscle. Gen. Physiol. Biophys. **6,** 109—112

Cazenave J.-P., Hemmendinger S., Beretz A. (1983): L'agrégation plaquettaire: outil d'investigation clinique et d'étude pharmacologique. Methodologie. Ann. Biol. Clin. (Paris) **41,** 167—179

Chang J., Musser J. H., McGregor H. (1987): Phospholipase A_2: Function and pharmacological regulation. Biochem. Pharmacol. **36,** 2429—2436

Enyeart J J , Biagi B A , Mlinar B (1992) Preferential block of T-type calcium channels by neuroleptics in neural crest-derived rat and human C cell lines Mol Pharmacol **42,** 364 372

Eto R , Yajima M , Takeya K (1991) The inotropic action of thioridazine and chlorpromazine in isolated guinea-pig papillary muscles Proc 13th Annu Meeting Jpn Soc Biol Psychiat Jpn J Psychiat Neurol **45,** 758

Hishinuma S Hongo J Uchida M K , Kurokawa M (1992) Haloperidol differentiates smooth muscle contractions induced by release of intracellularly stored Ca and by influx of extracellular Ca Gen Pharmacol **23,** 211 215

Kodavanti U P , Mehendale H M (1990) Cationic amphiphilic drugs and phospholipid storage disorder Pharmacol Rev **42,** 327 354

Kostyuk P G , Krishtal O A Pidoplichko V I (1981) Intracellular perfusion J Neurosci Meth **4,** 201 210

LaBella F S (1991) Molecular basis for binding promiscuity of antagonist drugs Biochem Pharmacol **42,** (Suppl) S1—S8

Liem L K Tewari K , Song Y , Simard J M (1994) The effect of papaverine on calcium channels in cerebrovascular smooth muscle Abstr XIIth Intern Congress Pharmacol Can J Physiol Pharmacol **72,** (Suppl 1), 579

Mannhold R (1988) Molecular pharmacology of calcium antagonists In Recent Advances in Receptor Chemistry (Eds C Melchiore, M Giannella) pp 117 171, Elsevier Science Publishers Amsterdam

Nishi K Akaike N Oyama, Y Ito H (1983) Actions of calcium antagonists on calcium currents in *Helix* neurons specifity and potency Circ Res **52,** (Pt 2) 1-53-1-59

Nosal' R , Jancinova V , Petrikova M (1994) Chloroquine and blood platelet functions in vitro Abstr XIIth Intern Congress Pharmacol Can J Physiol Pharmacol **72,** (Suppl 1) 140

Nosal' R , Jancinova V Petrikova M (1995) Chloroquine inhibits stimulated platelets at the arachidonic acid pathway Thromb Res **77,** 531 542

Pandey S C Davis J M , Schwertz D W , Pandey G N (1991) Effect of antidepressants and neuroleptics on phosphoinositide metabolism in human platelets J Pharmacol Exp Ther **256,** 1010—1018

Poda G , Tanchuk V (1994) The main cellular signaling systems and molecular similarity of chemical substances Abstr XIIth Intern Congress Pharmacol Can J Physiol Pharmacol **72,** (Suppl 1) 214

Prokopenko R , Zaitsev L , Mogilevich S Tetko I (1994) Non-specific actions of various drugs on smooth muscle Abstr XIIth Intern Congress Pharmacol Can J Physiol Pharmacol **72,** (Suppl 1) 581

Prokopenko R Zaitsev L , Mogilevich S Poda G , Naydyenova I Batrak G , Degtyar V , Luik A (1995) Inhibition of calcium-dependent cellular reactions by antagonist drugs which possess common pharmacophore Abstr First Europ Congress Pharmacol Pharmacol Res **31,** (Suppl), 207

Raeburn D , Gonzola R A (1988) CNS disorders and calcium antagonists Trends Pharmacol Sci **9,** 117 119

Seth P , Maitra K K , Ganguly D K (1991) Haloperidol on rat phrenic hemidiaphragm Arch Int Pharmacodyn Ther **310,** 87—93

Smith E A , Smith J L , Wall D (1990) The effect of calcium antagonists on ADP-induced activation of human platelets Aust N Z J Med **20,** (Suppl 1) 398

Tetsuyuki N. (1989): Actions of some drugs on skinned smooth muscle preparations. Gen. Pharmacol. **20,** 123–131

Tsukahara T., Wakatabe H., Ishigooka J., Miura S. (1991): Effect of neuroleptics on PI response following thrombin stimulation of human platelets. Proc. 13th Annu. Meeting Jpn. Soc. Biol. Psychiat. Jpn. J. Psychiat. and Neurol. **45,** 743

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