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Differences between haloperidol- and pimozide-induced withdrawal syndrome: a role for Ca^{2+} channels

Lucyna Antkiewicz-Michaluk, Beata Karolewicz, Jerzy Michaluk, Jerzy Vetulani *

Institute of Pharmacology, Polish Academy of Sciences, 31-343 Kraków, Poland

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Abstract

We investigated the behavioral and biochemical events appearing in rats after withdrawal for 24 h or 8–12 days from two classical neuroleptics, haloperidol and pimozide. The neuroleptics were given for 14 days alone or shortly after injection of the Ca^{2+} channel blocker nifedipine. We have found that withdrawal effects after haloperidol and pimozide were different. After haloperidol treatment we observed an increase in cortical Ca^{2+} channel and limbic dopamine D_1 receptor density and an increase in spontaneous motor activity and apomorphine-induced hyperactivity and stereotypy. In contrast no biochemical changes were observed during pimozide withdrawal, and locomotor activity and responses to apomorphine were depressed. Co-administration of nifedipine with haloperidol prevented the observed biochemical and behavioral symptoms of withdrawal. Nifedipine administration did not change the depressant effects of pimozide. Our results suggest that the voltage-dependent Ca^{2+} channel is involved in the observed withdrawal syndrome of neuroleptics, and that the absence of this syndrome after pimozide may be related to its considerable Ca^{2+} channel-blocking properties.

Keywords: Neuroleptic withdrawal; Ca^{2+} channel blockade; Haloperidol; Pimozide; Nifedipine; Apomorphine

1. Introduction

Chronic treatment of rodents with neuroleptics leads to development of tolerance, vacuous oral movements and – on withdrawal – supersensitivity of the dopamine system, reflected in an increase in dopamine receptor density in the striatum and mesolimbic areas (but not in the hippocampus), and increased spontaneous and apomorphine-induced locomotor activity and apomorphine-induced stereotypy (see Muller and Seeman, 1977, for review). These responses following prolonged administration of neuroleptics, particularly haloperidol, were investigated extensively as they are thought to be a model of the tardive or acute dyskinesia which develops after several months in patients treated with neuroleptics. The early theory that striatal dopamine receptor supersensitivity causes tardive dyskinesia has now given way to the hypothesis of multiple neurotransmitter system involvement (Jeste and Caligiuri, 1993). It has, however, been well established that long-term neuroleptic treatment also affects systems other than dopaminergic neurotransmitter systems. Thus, rats withdrawn from haloperidol appear to develop noradrenergic supersensitivity as they show increased motor responses to clonidine (Dustan and Jackson, 1976) and increased specific binding of an α_1-adrenoceptor ligand, [^{3}H]WB-4101 ([^{3}H]2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride), to cortical (but not striatal) membrane preparations (Muller and Seeman, 1977). They also seem to have suppressed muscarinic inhibitory mechanisms, which is reflected by an increased motor response to atropine (Dustan and Jackson, 1977) and reduction of K^+-induced acetylcholine release in the striatum (Friedman et al., 1990).

Changes in dopaminergic and non-dopaminergic systems observed on withdrawal after prolonged administration of neuroleptics is one of the accumulating examples of adaptive changes brought about by chronic treatment with drugs. Our earlier studies on adaptive changes induced by chronic administration of morphine (Antkiewicz-Michaluk et al., 1990b), electrocon-

* Corresponding author. Institute of Pharmacology, Polish Academy of Sciences, Smetna 121-343 Kraków, Poland. Tel.: +48 12 374022; fax: +48 12 374500; e-mail: nfvetula@cyf-kr.edu.pl.

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vulsive treatment (Antkiewicz-Michaluk et al., 1990a) and antidepressants (Antkiewicz-Michaluk et al., 1991) suggested that unimpaired function of Ca^{2+} channels is necessary for the development of some kinds of adaptive changes. Because of that it seemed of interest to investigate the effect of Ca^{2+} blockade on the effect of haloperidol withdrawal.

As we intended to investigate the effect of Ca^{2+} channel blockade on the haloperidol-induced withdrawal syndrome, it seemed of interest to investigate also the syndrome observed after withdrawal of a neuroleptic which itself has Ca^{2+} channel antagonistic properties: pimozide (Gould et al., 1983). Similarly to haloperidol, pimozide is regarded as a typical neuroleptic, but it differs from haloperidol in its chemical structure and, in contrast to haloperidol, was reported to be effective also in combating negative symptoms (Feinberg et al., 1988) and to produce little or no dyskinesia (McGuire et al., 1994). Moreover, it was found to be particularly effective in delusions of various nature (Driscoll et al., 1993; McGuire et al., 1994).

2. Materials and methods

2.1. Animals and treatment

The subjects were male Wistar rats, of initial weight 220–240 g, kept under standard laboratory conditions, eight to a large animal cage, with free access to standard laboratory food and tap water, at room temperature (~ 22°C) with a natural day-night cycle. The experiments were carried out between 10:00 h and 15:00 h.

Drugs

Neuroleptics, haloperidol (Sigma) 1 mg/kg i.p. and pimozide (RBI) 4 mg/kg i.p., were administered daily for 15 days. Nifedipine (Polfa) 5 mg/kg i.p. was given chronically, always 15 min before neuroleptic or placebo injection. The injections were given as a suspension in 1% Tween 80.

Apomorphine (Sandoz) 1 mg/kg i.p., was dissolved in 0.9% NaCl solution and given immediately before the behavioral tests.

Solutions of all drugs were prepared immediately before use and kept in dark bottles.

The animals were tested behaviorally or killed by decapitation 24 h or 8–12 days after the last injection.

2.2. Behavioral tests

Locomotor activity

Locomotor activity was measured in square photoresistor actometers with two crossed light beams, in which interruptions of a light beam were counted (Bednarczyk and Vetulani, 1977). The rats were placed into actometers individually for 30 min, and counting commenced immediately after introduction of the animals.

Stereotyped behavior

Stereotypy was assessed by continuing observation of the animals for 30 min. The degree of stereotypy was assessed according to an arbitrary scale (Table 1).

2.3. Membrane preparation and receptor binding assay

The experiment was carried out as described previously (Antkiewicz-Michaluk et al., 1990b). The brain was rapidly removed after decapitation, placed on an ice-chilled porcelain plate, and the cerebral cortices were dissected. The tissues were homogenized using a Polytron disintegrator (setting 4, 10 s) at 0°C in 20 volumes 50 mmol/l Tris-HCl buffer, pH 7.6. The cortex from each animal was homogenized separately.

The homogenate was centrifuged at 0°C and $1000 \times g$ for 10 min, the supernatant was decanted and recentrifuged at 0°C and $25000 \times g$ for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The pellet thus obtained (fraction P_2 of Whittaker and Barker, 1972) was stored at −18°C for no longer than 48 h. For incubation it was reconstituted in the Tris-HCl buffer to give a final protein concentration (measured according to Lowry et al., 1951) of approximately 1.2 mg/ml.

The incubation (vide infra) was carried out in duplicate, in a shaking water bath, at 25°C for 30 min. Addition of the radioligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 ml portions of ice-cold incubation buffer and placed in plastic scintillation minivials. Bray's fluid (3 ml) (Bray, 1960) was added and the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter.

In all experiments specific binding was defined as the difference between total and unspecific binding, and was expressed in fmol/mg protein.

[^{3}H]Nitrendipine displacement by neuroleptics in vitro

The cerebral cortex of naive animals was used. The incubation mixture (final volume 550 μl) consisted of 450 μl membrane suspension, 50 μl of a [^{3}H]nitrendipine solution (0.8–1 nM) and 50 μl buffer containing five concentrations of nifedipine (0.1 nM to 10 μM), and seven concentrations (1 nM to 100 μM) of haloperidol, pimozide, or spiperone. For measuring unspecific binding, nifedipine in a final concentration of 10 μM was present.

[^{3}H]Nitrendipine binding sites density changes

Tissues from animals undergoing chronic treatment and killed at appropriate times were used. The radioli-

gand, [^{3}H]nitrendipine (NEN, specific activity 78.3 Ci/mmol), was prepared in the dark in six concentrations (0.05–3 nM) in the buffer. The incubation mixture (final volume 550 μl) consisted of 450 μl membrane suspension, 50 μl of a [^{3}H]nitrendipine solution and 50 μl buffer without (total binding) or with (unspecific binding) nifedipine (final concentration 10 μM).

Limbic dopamine D_1 receptor

Limbic structures (containing the olfactory tubercle, preoptic area, nucleus accumbens, septum, amygdala and limbic cortex) were dissected rapidly after decapitation and placed immediately on solid CO_2. The tissue was stored at −70°C until use, when it was homogenized in 40 volumes of an ice-cold 50 mM Tris-HCl buffer, pH 7.4, using a Polytron disintegrator. The homogenate was centrifuged at $1000 \times g$ for 15 min, the supernatant was decanted and recentrifuged at $25000 \times g$ for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The final pellet (fraction P_2 of Whittaker and Barker, 1972) was used for binding studies. For incubation it was reconstituted in the Tris-HCl buffer pH 7.4 for the dopamine D_1 receptor and Tris-HCl buffer pH 7.1 + ions (120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbic acid) for the dopamine D_2 receptor to obtain a final protein concentration (measured according to Lowry et al., 1951) of approximately 0.3–0.4 mg/ml.

The radioligand, [^{3}H]SCH-23390 ([^{3}H](R +)7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride, NEN, specific activity 85.5 Ci/mmol), was prepared in six concentrations from 0.06–2.0 nM. The incubation mixture (final volume 550 μl) consisted of 450 μl membrane suspension, 50 μl of [^{3}H]SCH-23390 solution and 50 μl buffer without (total binding) or with (unspecific binding) cold SCH-23390 (final concentration 5 μM). All assays were performed in duplicate and incubation was in a shaking water bath, at 30°C for 60 min.

2.4. Statistics

The results were evaluated by Scatchard analysis for assessment of B_{max} and K_D values. The significance of differences was assessed with one way analysis of variance, followed, if required, by Fisher's Least Significant Difference (LSD) test.

3. Results

3.1. Neuroleptic affinity to [^{3}H]nitrendipine binding sites

Pimozide effectively displaced [^{3}H]nitrendipine from its cortical binding sites in a nanomolar concentration range. Its low IC_{50} and the shape of the curve suggested competitive binding. Haloperidol and spiperone were more than two orders of magnitude less potent, and sulpiride was practically ineffective in this respect (Fig. 1).

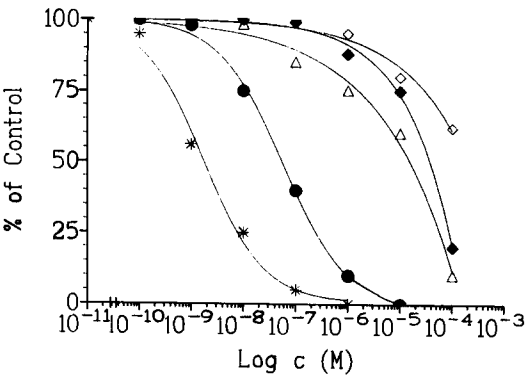

Fig. 1. Displacement of [^{3}H]nitrendipine by neuroleptics from cerebral cortical synaptosomes. The IC_{50} values for various drugs were: nifedipine (asterisks) 5 nM, pimozide (closed circles) 75 nM, haloperidol (open triangles) 10000 nM, spiperone (closed diamond) 20000 nM, sulpiride (open diamond) > 100000 nM.

3.2. Ca^{2+} channels

Treatment effect

24 hours after the last of 15 daily treatments with haloperidol the density of [^{3}H]nitrendipine binding sites was significantly increased, by 55%, without a change in K_D value (Fig. 2). The treatment with pimozide did not affect the density of [^{3}H]nitrendipine binding sites but significantly elevated the K_D value (Fig. 3). Treatment with nifedipine alone produced a reduction in [^{3}H]nitrendipine binding sites, but the effect was variable, being approx. 20% (N.S.) in one experiment and approx. 40% ($P < 0.05$) in another (Figs. 2 and 3). The augmenting effect of haloperidol was completely pre-

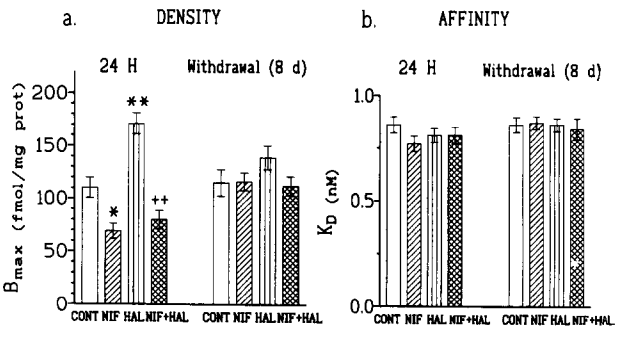

Fig. 2. The effect of chronic administration of haloperidol, nifedipine, or their combination on cortical [^{3}H]nitrendipine binding sites, 24 h or 8 d after the last injection. (a) Density. (b) Affinity. CONT – saline control, NIF – nifedipine, 5 mg/kg daily, HAL – haloperidol, 1 mg/kg daily for 14 days. $^*P < 0.05$; $^{**}P < 0.01$ (difference from control), $^+P < 0.05$; $^{++}P < 0.01$ (difference from haloperidol group).

vented by co-administration of nifedipine (Fig. 2). The depression of the affinity of [^{3}H]nitrendipine binding sites by pimozide was unaffected by co-administration of nifedipine (Fig. 3).

Withdrawal effect

8 days after cessation of drug injections, the parameters of [^{3}H]nitrendipine binding in the haloperidol-treated group were identical in the control and drug-injected groups (Fig. 2), but the parameters of [^{3}H]nitrendipine binding sites in the pimozide-treated group were similar to those observed 24 h after the end of the treatment (Fig. 3).

3.3. Limbic dopamine D_1 receptors

Treatment effects

The density of [^{3}H]SCH-23390 binding sites was significantly increased (by approx. 40%) in the haloperidol-treated group, while no changes were observed in the groups receiving pimozide (Fig. 5) or nifedipine (Figs. 4 and 5). The augmenting effect of haloperidol treatment was effectively inhibited by co-administered nifedipine (Fig. 4). No changes in K_D were observed in any group. Nifedipine treatment did not produce significant changes in the density of [^{3}H]SCH-23390 binding sites.

Withdrawal effects

The density of [^{3}H]SCH-23390 binding sites in haloperidol-treated group remained significantly augmented (by 28%) after 12 days of withdrawal. No differences from the controls were observed in the remaining experimental groups (Figs. 3 and 4).

3.4. Spontaneous locomotor activity

Treatment effects

Nifedipine alone produced a reduction in spontaneous locomotor activity by 25–40%, but this effect did not reach statistical significance.

Spontaneous locomotor activity was significantly augmented (by 76%) in rats receiving haloperidol 24 h after the last injection, and this effect was abolished by co-administration of nifedipine. In contrast, the locomotor activity of rats receiving pimozide chronically (alone or with nifedipine) was depressed 24 h after the last injection by approx. 40%, but this effect did not reach statistical significance ($P < 0.1$).

Withdrawal effects

No effects of nifedipine treatment on spontaneous locomotor activity were observed 8 days after the last injection. The activity of the haloperidol-treated group was significantly increased (by 84%) during the withdrawal period, but no increase was observed in the group receiving haloperidol with nifedipine. In the pimozide- and pimozide and nifedipine-treated groups locomotor activity during withdrawal was non-significantly depressed.

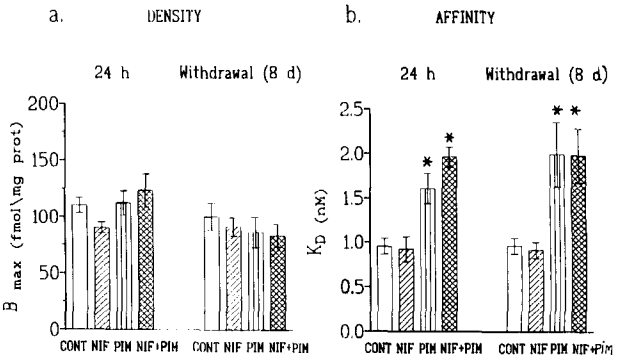

Fig. 3. The effect of chronic administration of pimozide, nifedipine, or their combination on cortical [^{3}H]nitrendipine binding sites, 24 h or 8 days after the last injection. (a) Density. (b) Affinity. PIM – pimozide, 4 mg/kg daily for 14 days. For other explanations see Fig. 2.

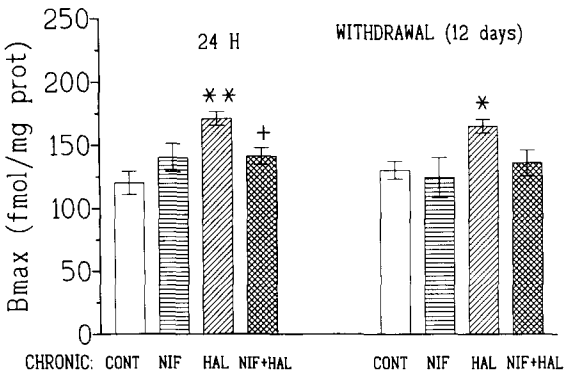

Fig. 4. The effect of chronic administration of haloperidol, nifedipine, or their combination on limbic [^{3}H]SCH-23390 binding site density, 24 h or 12 d after the last injection. The mean K_D values remained unchanged and were 0.65 ± 0.08 nM. For other explanations see Fig. 2.

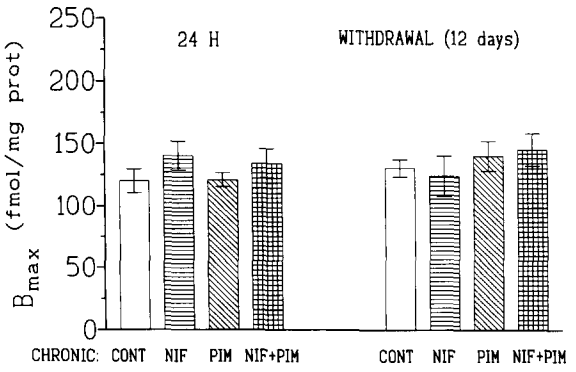

Fig. 5. The effect of chronic administration of pimozide, nifedipine, or their combination on limbic [^{3}H]SCH-23390 binding site density, 24 h or 12 days after the last injection. The mean K_D values remained unchanged and were 0.70 ± 0.09 nM. For other explanations see Figs. 2 and 3.

3.5. Responsiveness to apomorphine

Apomorphine hyperactivity

Treatment effects. The motor response to apomorphine was significantly augmented in the haloperidol-treated group (by 52% in comparison to apomorphine control), but remained at the control level in the group in which haloperidol was combined with nifedipine (Table 1). The response to apomorphine was significantly reduced in rats pretreated with pimozide, and co-administration of nifedipine did not alter the pimozide effects (Table 2).

Withdrawal effects. Haloperidol effects after 8 days of withdrawal were similar to those observed 24 h after the last dose: the apomorphine-enhanced locomotor activity was significantly augmented in the haloperidol-treated group (by 80%), but the effects were prevented by co-administration of nifedipine (Table 1). In the group withdrawn from pimozide the response to apomorphine was still significantly reduced (by 50%), but the reduction was smaller (30%) and did not reach significance in the group receiving pimozide after nifedipine (Table 2).

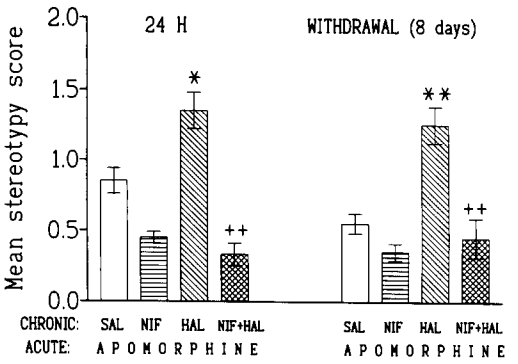

Fig. 6. The effect of chronic administration of haloperidol, nifedipine, or their combination on apomorphine-induced stereotypy, 24 h or 8 days after the last injection. For other explanations see Fig. 2.

Apomorphine stereotypy

Treatment effects. Nifedipine treatment caused a reduction of the stereotypy score (by 40%), which did not reach the significance level ($P < 0.1$), while the stereotypy response in the haloperidol-treated group was significantly augmented (by 60%). The group treated with the combination of nifedipine and haloperidol

Table 1
Spontaneous and apomorphine-stimulated motor activity 24 h and 8 days after the end of chronic treatment with haloperidol

	Treatment (24 h)		Withdrawal (8 days)	
	SAL	APO	SAL	APO
Control	125 ± 25 (10)	198 ± 34 (10)	136 ± 25 (10)	166 ± 29 (10)
NIF	76 ± 19 (10)	120 ± 21 (10)	130 ± 21 (10)	184 ± 36 (10)
HAL	220 ± 46 (10) [a]	300 ± 39 (10) [a]	250 ± 38 (10) [a]	300 ± 56 (10) [a]
NIF + HAL	72 ± 20 (11) [b]	172 ± 23 (12) [b]	159 ± 25 (10) [b]	195 ± 36 (10) [b]
	$F(3/38) = 7.39$)	$F(3/39 = 5.06$	$F(3/37) = 4.47$	$F(3/37) = 2.75$
	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$

The data are means ± S.E.M. of beam crossings for 30 min. The numbers in parentheses denote group size. SAL: saline; APO: apomorphine. Control: the group receiving saline during pretreatment; NIF: nifedipine, 5 mg/kg i.p. daily for 14 days; HAL: haloperidol 1 mg/kg i.p. for 14 days; NIF + HAL: combined treatment with nifedipine and haloperidol for 14 days. [a] Significantly different from the control group ($P < 0.05$, LSD test), [b] significantly different from group pretreated with haloperidol alone ($P < 0.05$).

Table 2
Spontaneous and apomorphine-stimulated motor activity 24 h and 8 days after the end of chronic treatment with pimozide

	Treatment (24 h)		Withdrawal (8 days)	
	SAL	APO	SAL	APO
Control	117 ± 21 (10)	168 ± 29 (10)	98 ± 19 (10)	169 ± 21 (10)
NIF	88 ± 18 (10)	132 ± 25 (10)	91 ± 16 (10)	168 ± 24 (10)
PIM	73 ± 10 (10)	83 ± 17 (10) [a]	77 ± 11 (10)	93 ± 15 (10) [a]
NIF + PIM	71 ± 13 (10)	80 ± 17 (10)	73 ± 9 (10)	120 ± 28 (10)
	$F(3/37) = 1.87$	$F(3/37) = 2.65$	$F(3/37) = 0.59$	$F(3/37) = 3.16$
	N.S.	$P < 0.05$	N.S.	$P < 0.05$

The data are means ± S.E.M. of beam crossings for 30 min. The numbers in parentheses denote group size. SAL: saline; APO: apomorphine. Control: the group receiving saline during pretreatment; NIF: nifedipine, 5 mg/kg i.p. daily for 14 days; PIM: pimozide, 4 mg/kg i.p. for 14 days; NIF + PIM: combined treatment with nifedipine and pimozide for 14 days. [a] Significantly different from the control group ($P < 0.05$, LSD test).

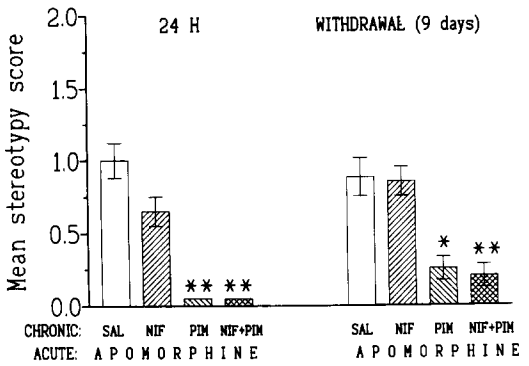

Fig. 7. The effect of chronic administration of pimozide, nifedipine, or their combination on apomorphine-induced stereotypy, 24 h or 8 days after the last injection. For other explanations see Figs. 2 and 3.

displayed the stereotypy response at the level similar to that observed in the nifedipine-treated group (Fig. 6). In the group receiving pimozide (with or without nifedipine), no stereotypy response was observed 24 h after the end of the treatment (Fig. 7).

Withdrawal effects. The nifedipine-treated group did not differ significantly from the control (−30%), but the group withdrawn from haloperidol showed the augmented response to apomorphine, even stronger than 24 h after the end of the treatment (100%). The response of the group receiving haloperidol together with nifedipine remained at the level observed in rats receiving nifedipine alone (Fig. 6).

9 days after cessation of pimozide administration the stereotypy response to apomorphine was still significantly reduced (by 73%); co-administration of nifedipine did not alter the pimozide effect (Fig. 7).

4. Discussion

The present results indicate that behavioral and biochemical effects related to neuroleptic withdrawal are different in rats treated chronically with haloperidol and with pimozide, and that the withdrawal effects are attenuated in animals receiving neuroleptics concurrently with a Ca^{2+} channel blocker, nifedipine.

Haloperidol and pimozide are regarded as effective typical neuroleptics but belong to different chemical groups and show several differences in clinical action. Pimozide, a diphenylbutylpiperidine derivative, and haloperidol, a butyrophenone, are both antagonists of dopamine D_2 receptors, and bind equally to dopamine D_2 and D_3 receptors (Freedman et al., 1994), but show differences in their affinity to other receptors. Thus in addition to being a potent antagonist of dopamine receptors, pimozide is also an effective antagonist of various subtypes of 5-HT receptors, particularly of the rat cloned 5-HT_7 receptor, to which it had the highest affinity ($K_i = 0.5$ nM) of all the typical antipsychotic agents, and is approx. 500 times more potent than haloperidol (Roth et al., 1994). Pimozide is also a potent Ca^{2+} channel antagonist, which allosterically modulates the L-type Ca^{2+} channel (Gould et al., 1983). Our results confirm that pimozide effectively displaces [^{3}H]nitrendipine from its cortical binding sites and demonstrated that the effect of haloperidol is approx. 150 times weaker. Recent studies showed that pimozide blocks more effectively than haloperidol the N- and P-type Ca^{2+} channels (Sah and Bean, 1994) and T-type Ca^{2+} channels also (Enyeart et al., 1993). Diphenylbutyropiperidines were also described as potent inhibitors of calmodulin (Weiss et al., 1982) and pimozide was frequently used in this capacity in biochemical experiments (Izosaki et al., 1994; Rodriguez-Medina et al., 1993).

The classical study of Janssen et al. (1968) described pimozide as acting similarly to haloperidol but having a much longer duration of action. The differences between haloperidol and pimozide appear after chronic treatment, and this is particularly evident in clinical practice. While haloperidol is known as very effective, but particularly in alleviating the positive symptoms of schizophrenia, and causing tardive dyskinesia after prolonged administration, pimozide was described as superior to haloperidol in alleviating the negative symptoms of schizophrenia (Opler and Feinberg, 1991). Pimozide produces less tardive dyskinesia, and is particularly effective in delusional disorders such as erotomania (McGuire et al., 1994) and delusional parasitosis (Driscoll et al., 1993). The Ca^{2+}-antagonistic properties of pimozide were also employed clinically: the drug was found effective in delirium secondary to hypercalcemia (Mark et al., 1993) and in preclinical studies it was found to prevent muscle damage induced by high intracellular Ca^{2+} (Beitner and Lilling, 1993). It was also described as a potent Ca^{2+} channel antagonist in the heart (Enyeart et al., 1990). In the light of the interesting profile of biochemical and clinical actions of pimozide it is rather surprising that it has been much less investigated than haloperidol (in the Medline Search for January to November 1994 only 39 papers reporting on pimozide were found, compared with 401 on haloperidol) and no paper on pimozide withdrawal with the exception of our preliminary note (Mamczarz et al., 1994) was published to the best of our knowledge. In contrast, effects of withdrawal from chronic or subchronic administration of haloperidol and other neuroleptics have been studied extensively (see Introduction).

One of the main findings of the present study is that haloperidol and pimozide produce different biochemical effects both during treatment (measured 24 h after the last dose) and 8–12 days after withdrawal. Chronic

treatment with haloperidol and pimozide affected L-type Ca^{2+} channels in opposite ways – haloperidol up-regulated the nitrendipine binding sites, significantly increasing their density with no changes in affinity, while pimozide down-regulated the Ca^{2+} channels by decreasing affinity without changing the density. While the haloperidol-induced up-regulation disappeared within 7 days, the effect of pimozide was long-lasting. The present findings concerning pimozide correspond well with our in vitro findings that pimozide displaces [^{3}H]nitrendipine from its cortical binding sites much more potently than haloperidol and indicate that the regulation is persistent. Haloperidol also differed from pimozide in its action on limbic dopamine D_1 receptors: only the first compound produced persistent up-regulation of these receptors.

An even greater contrast in the action of haloperidol and pimozide was observed in behavioral tests. Although single doses of pimozide produce effects similar to those of haloperidol (Janssen et al., 1968), after chronic administration their behavioral action is different. While 24 h after the last dose haloperidol produced a significant increase in spontaneous locomotor activity and apomorphine-induced hyperactivity and stereotypy, and these potentiating effects after 8 days of withdrawal were maintained without change in intensity, pimozide instead depressed spontaneous locomotor activity and significantly blocked the actions of apomorphine both 24 h and 8 days after withdrawal. The behavioral responses to apomorphine 3 weeks after the end of administration of both neuroleptics returned to normal (results not shown). Thus, while haloperidol withdrawal produced a transient up-regulation of cortical Ca^{2+} channels and longer lasting up-regulation of limbic dopamine D_1 receptors and produces behavioral responses opposite to those produced by a single dose of the drug in naive animals, pimozide after withdrawal continues to down-regulate Ca^{2+} channels and antagonize the effects of apomorphine. The present behavioral results agree with results of our preliminary study on the effect of haloperidol and pimozide withdrawal on open-field behavior of pairs of rats, from which we reported that rats withdrawn from haloperidol show higher explorative activity, while those withdrawn from pimozide show significantly decreased exploration and social contacts (Mamczarz et al., 1994). It is at present unknown whether 12 days after pimozide withdrawal the drug remains bound to cerebral receptors. The increase in K_D of [^{3}H]nitrendipine binding sites could suggest the presence of pimozide at these sites, while the lack of changes of binding parameters of dopamine D_1 receptors (and also of dopamine D_2 receptors; Antkiewicz-Michaluk et al., unpublished) indicates no presence of pimozide at dopamine receptors 12 days after withdrawal. In human studies, it was found that after withdrawal from chronic treatment with several neuroleptics, the cerebral receptors are free of neuroleptics within 5–8 days (Baron et al., 1989). Also in human studies, it was found that, in the steady state, after subchronic administration of haloperidol decanoate, the plasma level of the neuroleptic is higher than after a single dose but its elimination is accelerated. In contrast, the elimination rate of pimozide is not changed after chronic administration (Jørgensen, 1986). No studies on the rate of disappearance of pimozide and haloperidol from the rat brain during the withdrawal period are available. However, our unpublished studies indicating that amphetamine responses are equally potentiated during withdrawal from haloperidol and pimozide suggest that a pharmacokinetic factor did not play a decisive role in the observed differences in the action of those neuroleptics reported in this study.

The present findings that nifedipine prevents the development of withdrawal symptoms, particularly expressed in the group receiving haloperidol, seem interesting. They are in line with our previous findings which indicate that blockade of voltage-dependent Ca^{2+} channels during chronic administration of several compounds prevents the development of drug-related adaptive changes (Antkiewicz-Michaluk et al., 1993a,b, 1994), and with our preliminary findings (Mamczarz et al., 1994). Analogous results were reported by Whittington et al. (1991) for the ethanol abstinence syndrome in mice: the authors found that chronic ethanol treatment elevates the density of Ca^{2+} channels in the brain and that co-administration of nitrendipine with ethanol prevents behavioral and biochemical effects of the abstinence.

Prevention of the withdrawal syndrome after neuroleptics may be of clinical interest, particularly as it is believed that tardive dyskinesia results from supersensitivity of dopamine receptors (Seeman, 1985). In addition, the supersensitivity to dopamine may result from an increase in the density of cerebral voltage-dependent Ca^{2+} channels (Antkiewicz-Michaluk et al., 1990a,b). As haloperidol treatment resulted in a transient increase in [^{3}H]nitrendipine binding site density, a part of the supersensitivity to apomorphine after haloperidol withdrawal might result from this effect also.

It was suggested that neuroleptic-induced dopaminergic supersensitivity is caused by changes in dopamine D_2 receptors, and that antagonism to dopamine D_1 receptors prevents the development of withdrawal supersensitivity. Thus, sulpiride, a specific dopamine D_2 antagonist, produces dopaminergic supersensitivity on withdrawal when given alone (Frussa Filho and Palermo Neto, 1990), but not when given with the dopamine D_1 receptor antagonist, SCH-23390 (Dall'Olio et al., 1990). However, similarly to haloperidol, pimozide is a rather

specific dopamine D_2 receptor antagonist (Hyttel et al., 1985). Therefore, the lack of withdrawal syndrome after pimozide cannot be attributed to its action on dopamine D_1 dopamine receptors. We suggest that it might result from its potent Ca^{2+} channel-blocking properties, while haloperidol and sulpiride, much less potent in this respect, at the concentration present in the brain cannot block Ca^{2+} channels and produce the withdrawal syndrome. However, combined treatment with a Ca^{2+} channel blocker and a neuroleptic lacking intrinsic Ca^{2+} channel-blocking properties prevented the development of biochemical and behavioral signs of withdrawal. The augmenting effect of haloperidol treatment on [^{3}H]nitrendipine and [^{3}H]SCH-23390 binding site density was effectively inhibited by co-administered nifedipine, as was behavioral supersensitivity: the motor and stereotypy response to apomorphine remained at the control level in this group.

In contrast to interaction with haloperidol, co-administration of nifedipine with pimozide virtually did not change the effects of the neuroleptic. In fact, during the withdrawal period the pimozide-treated group was still behaviorally depressed. We suggest that the blockade of Ca^{2+} channel by pimozide was strong enough to allow for any additional effect of nifedipine.

The present results suggest that Ca^{2+} channel blockers may be useful in combination with neuroleptics lacking potent Ca^{2+} channel-blocking properties, as they may counteract the development of dopaminergic supersensitivity. Similar conclusions are suggested by results of Pucilowski and Kostowski (1988) and Pucilowski and Eichelman (1991), who reported that diltiazem prevents some effects of haloperidol withdrawal. A clinical experiment might also answer the intriguing question as to whether or not the unique favorable effect of pimozide in counteracting the negative symptoms of schizophrenia (Feinberg et al., 1988) is related to its Ca^{2+} channel-blocking properties.

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