DEVELOPMENT OF TOLERANCE TO THE STIMULATORY EFFECT OF NEUROLEPTIC BUTYROPHENONES ON PITUITARY-ADRENAL ACTIVITY IN RATS

TACHIO AIMOTO, MORIO KAIDA, KATSUJI NUMAZAKI, YUKINORI MASUDA, AND TOSHIRO MURATA

Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka 422, Japan

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The effects of repeated administration of haloperidol (HAL) and clofluperol (CLO) on the responsiveness of pituitary-adrenal system to these drugs were studied in rats. Chronic treatment of animals with a daily *i.p.* dose of 5 μ mol/kg of the drugs resulted in markedly attenuated responses of plasma corticosterone (CS) to challenge of the same dose of the same drug as compared to the acute treatment which caused a large increase in the plasma CS levels. Animals receiving a chronic HAL treatment showed a complete cross-tolerance to the stimulatory effect of CLO and a nearly complete cross-tolerance to that of chlorpromazine.

The adrenal cortices of animals tolerant to HAL responded normally to exogenous ACTH. This result indicates that the tolerance to the stimulatory action of the butyrophenones may occur in the hypothalamo-pituitary axis.

Keywords—neuroleptic butyrophenones; haloperidol; clofluperol; chlorpromazine; pituitary-adrenocortical activity; plasma corticosterone; repeated administration; tolerance

INTRODUCTION

In a previous report,¹⁾ we have shown that acute treatment of rats with haloperidol (HAL), a typical neuroleptic butyrophenone, and some other butyrophenones resulted in marked increases in plasma and adrenal corticosterone (CS) levels, and that the release of ACTH from the pituitary was necessary for the action. Recently, Giraud *et al.* demonstrated that a single HAL administration to rats caused a large increase of plasma ACTH.²⁾ In addition, chronic treatment of rats with droperidol was reported to cause an increased production of CS in adrenals.³⁾ When rats were treated chronically with a relatively high dose of HAL or clofluperol (CLO), an elevation of plasma CS was observed.⁴⁾ Thus, chronic treatment with the butyrophenones may bring about an increase in the basal activity of the adrenal cortex. However, the responsiveness of pituitary-adrenal system to the butyrophenones after chronic treatment with the drugs has not yet been

studied.

We, therefore, examined the effects of repeated administration of HAL and CLO on the responsiveness of pituitary-adrenocortical system to the drugs in rats. The present communication provides evidence for the development of tolerance to the stimulation of the system by the drugs following chronic treatment.

EXPERIMENTAL

HAL and CLO were generous gifts from Dai-nippon Pharmaceutical Co., Ltd.

Sixty to eighty days old female Wistar rats weighing 140 to 160 g at the beginning of the experiments were housed in a room maintained at $24 \pm 2^\circ\text{C}$ on a 12-h light-dark cycle (lights on at 6:00 a.m.) with food and water available *ad libitum*. All animals were handled daily for at least 5 days prior to an experimental procedure in order to minimize stress responses from handling and injections. To avoid possible variations related

to circadian rhythm, the drug treatment and sampling of blood by decapitation of the animals were carried out between 8:00 and 10:00 a.m., during which rat plasma CS was at its resting level as confirmed previously.¹⁾

In the acute treatment, rats received a single *i.p.* injection of 5 $\mu\text{mol/kg}$ of HAL or CLO dissolved in distilled water or an equivalent volume of the vehicle, and were killed 1 h later. In the chronic treatment, rats were given the same dose of the drugs or vehicle as in the acute treatment once a day for 1, 3, 5, 10 or 20 days. At specified time intervals after the last treatment, they were given 5 $\mu\text{mol/kg}$ or higher doses of HAL (*i.p.*), 5 $\mu\text{mol/kg}$ of CLO (*i.p.*), synthetic ACTH₁₋₂₄

(Cortrosyn®, Daiichi Pharmaceutical Co., Ltd.; 10 U/kg, *s.c.*) or vehicle (*i.p.*) and were killed 1 h later. In the cross-tolerance study, rats received a daily *i.p.* injection of 5 $\mu\text{mol/kg}$ of HAL or vehicle for 10 days. Twenty-four hours after the last treatment, they were challenged with an *i.p.* dose of CLO (5 $\mu\text{mol/kg}$), chlorpromazine (CPZ, Contomin®, Yoshitomi Pharmaceutical Co., Ltd.; 20 $\mu\text{mol/kg}$) or vehicle and were killed 1 h later.

Separation of plasma and determination of the plasma CS were carried out as described before.¹⁾

RESULTS

Fig. 1 shows the responses of pituitary-adrenal system to HAL as assessed by determining the changes in plasma CS levels 1 h after the *i.p.* administration of 5 $\mu\text{mol/kg}$ of the drug to rats which were pretreated with the same daily dose

FIG. 1. Effect of Repeated Administration of Haloperidol on Response of Plasma Corticosterone to the Drug in Rats

The repeated dose-groups of female Wistar rats received a daily *i.p.* injection of the drug (5 $\mu\text{mol/kg}$) at 8:00 a.m. for 1, 3, 5, 10 or 20 days. Then they were given the same dose of the drug 24 h after the last dose and killed by decapitation 1 h later. Two other groups were given a single *i.p.* dose of the drug or vehicle and killed 1 h later. Data represent the mean $\pm$ S.E. (vertical bars) for 5 to 9 animals.

□, control (vehicle); ▨, acute dose; ▩, repeated dose.

a) Significantly different from control at a *p* value of 0.05 or less. b) Significantly different from acute dose at a *p* value of 0.05 or less.

FIG. 2. Effect of Repeated Administration of Clofluperol on Response of Plasma Corticosterone to the Drug in Rats

The animals were treated with clofluperol as described in the legend to Fig. 1. Data represent the mean $\pm$ S.E. (vertical bars) for 5 to 7 animals.

□, control; ▨, acute dose; ▩, repeated dose.

a) Significantly different from control at a *p* value of 0.05 or less. b) Significantly different from acute dose at a *p* value of 0.05 or less.

of the drug *i.p.* for up to 20 days. An acute *i.p.* administration of the drug caused a marked elevation of plasma CS levels, as observed in our earlier work,¹⁾ while significant attenuations of the plasma CS responses to the drug were observed in the repeated dose-groups after as early as 3 days of treatment. Further prolongation of the treatment period did not alter the responsiveness of the pituitary-adrenal system. When rats were treated with HAL for 10 days, their plasma CS response to the drug decreased greatly by more than 85% of that of the acute dose-group. Thus, the repeatedly treated groups in this study showed the development of tolerance to the stimulatory effect of the drug on the pituitary-adrenocortical system. However, the responses of the system to the drug did not completely disappear even in the extremely tolerant animals; the plasma CS levels after challenging with the drug in those animals who received 5 to 20 days of treatment were still higher than those after a single vehicle administration to the control animals.

The same extent of response of plasma CS to CLO as that to HAL in chronically HAL-treated rats were also observed after the repeated administration of CLO to rats, as illustrated in Fig. 2.

We also examined the effects of repeated administration of HAL and CLO on adrenal

weights and the basal levels of plasma CS. As shown in Table I, the repeated HAL administration did not cause any changes in the relative adrenal weights, while CLO produced increases in those of the animals treated with the drug for 10 and 20 days. The repeated vehicle injection did not modify the basal levels of plasma CS. The mean plasma CS levels ($\mu\text{g}/100\text{ ml} \pm \text{S.E.}$) in the single and repeated vehicle groups were as follows: single group, 6.5 ± 0.4 (Fig. 1) and repeated groups, 6.0 ± 0.3 to 9.0 ± 1.1 (Table I, control). The repeated HAL challenge did not alter the basal plasma CS levels, while the repeated CLO treatment elicited a sustained elevation of the basal plasma CS levels.

Fig. 3 shows the effect of HAL challenge on the plasma CS levels during withdrawal from chronic treatment. One to five days after the cessation of chronic HAL treatment, a marked decrease in the response of plasma CS was observed by challenging with $5\text{ }\mu\text{mol/kg}$ of HAL as compared to the response in the acute treatment group. The tolerance observed soon after the last injection of a 10 days of treatment is thus demonstrable for at least 5 days after the termination of the treatment. But by 10 days after the termination, the plasma CS responsiveness to HAL appeared to show a recovery.

TABLE I. *Effects of Repeated Administration of Haloperidol and Clofluperol on Adrenal Weights and Basal Levels of Plasma Corticosterone in Rats*

	Drug	Days of treatment			
		3	5	10	20
Adrenal wt. (mg/100 g body wt.)	Control	28.4 ± 0.4	27.2 ± 1.1	27.5 ± 1.2	25.6 ± 0.9
	Haloperidol	28.7 ± 0.9	27.3 ± 1.1	25.7 ± 1.6	23.4 ± 0.7
	Clofluperol	29.5 ± 0.9	29.4 ± 1.3	$38.1 \pm 1.5^a)$	$36.6 \pm 1.7^a)$
Plasma corticosterone ($\mu\text{g}/100\text{ ml}$)	Control	8.1 ± 1.9	6.0 ± 0.3	7.0 ± 1.2	9.0 ± 1.1
	Haloperidol	6.1 ± 1.0	6.1 ± 0.2	7.2 ± 0.9	14.5 ± 2.3
	Clofluperol	$25.5 \pm 2.3^a)$	$15.6 \pm 2.1^a)$	$22.8 \pm 3.1^a)$	$15.0 \pm 0.8^a)$

The animals received a daily *i.p.* injection of the drug ($5\text{ }\mu\text{mol/kg}$) or vehicle for different periods of time indicated. Twenty-four hours after the last treatment, they were given *i.p.* the vehicle alone and killed by decapitation 1 h later. Data represent the mean $\pm$ S.E. for 5 to 6 animals.

a) Significantly different from the respective control at a *p* value of 0.01 or less.

To determine whether there was any response of the pituitary-adrenal system to higher doses

above 5 $\mu\text{mol/kg}$ of HAL, the effect of increasing the challenge dose of the drug up to 20 $\mu\text{mol/kg}$

FIG. 3. Persistence of Tolerance of Plasma Corticosterone Response to Haloperidol in Rats treated chronically with the Drug

The animals received a daily i.p. injection of haloperidol (5 $\mu\text{mol/kg}$) for 10 days. Then they were challenged with the same dose of the drug i.p. at the indicated times after the last dose and killed by decapitation 1 h later. Data represent the mean $\pm$ S.E. (vertical bars) for 6 to 10 animals. □, acute dose; ■, chronic dose.

a) Significantly different from acute dose at a p value of 0.001.

FIG. 4. Response of Plasma Corticosterone to Graded Doses of Haloperidol in Rats pretreated chronically with the Drug

The animals were given a daily i.p. injection of haloperidol (5 $\mu\text{mol/kg}$, ■) or vehicle (□) for 10 days. Then they were challenged with 5 $\mu\text{mol/kg}$ or larger doses of haloperidol or vehicle i.p. 24 h after the last treatment and killed by decapitation 1 h later. Data represent the mean $\pm$ S.E. (vertical bars) for 5 to 9 animals.

a) Significantly different from chronic vehicle at a p value of 0.01 or less. b) Significantly different from chronic haloperidol (vehicle challenge) at a p value of 0.05 or less.

TABLE II. Effects of Haloperidol, Clofluperol and Chlorpromazine on Plasma Corticosterone Response in Rats pretreated chronically with Haloperidol

Drug	Dose ($\mu\text{mol/kg}$)	Plasma corticosterone ($\mu\text{g}/100\text{ ml}$)			
		Chronic treatment			
		Vehicle		Haloperidol	
Control	—	7.0 $\pm$ 1.2	(5)	10.7 $\pm$ 1.4	(6)
Haloperidol	5	105.4 $\pm$ 9.0 ^{a)}	(9)	14.6 $\pm$ 2.3 ^{b)}	(9)
Clofluperol	5	117.7 $\pm$ 7.0 ^{a)}	(5)	14.1 $\pm$ 1.9 ^{b)}	(5)
Chlorpromazine	20	93.3 $\pm$ 12.9 ^{a)}	(6)	27.2 $\pm$ 3.3 ^{a,b)}	(8)

The animals were given a daily i.p. dose of 5 $\mu\text{mol/kg}$ of haloperidol or vehicle for 10 days. Then they were challenged with the drugs indicated above or vehicle i.p. 24 h after the last chronic treatment with haloperidol or vehicle and killed 1 h later. Data represent the mean $\pm$ S.E. for the number of animals indicated in parenthesis.

a) Significantly different from the respective control at a p value of 0.01 or less. b) Significantly different from the respective chronic vehicle at a p value of 0.01 or less.

on plasma CS levels was studied in rats pretreated with 5 $\mu\text{mol/kg}$ of HAL for 10 days. Fig. 4 shows the results. In tolerant rats to HAL, the plasma CS response to a challenge dose of 5 $\mu\text{mol/kg}$ was the same extent as that to a vehicle challenge. With a challenge dose of 10 $\mu\text{mol/kg}$, HAL produced a significant elevation of plasma CS, though its degree of elevation was much smaller than that produced by the acute HAL (5 $\mu\text{mol/kg}$) treatment in non-tolerant animals. Furthermore, a dose of 15 $\mu\text{mol/kg}$ caused an elevation of plasma CS levels to the same degree as that produced by the acute treatment of non-tolerant animals with

HAL (5 $\mu\text{mol/kg}$). Further increase of the challenge dose resulted in a stimulation of the pituitary-adrenal activity above that evoked by the acute treatment.

To determine whether there was a cross-tolerance to CLO and other non-butyrophenone neuroleptics such as CPZ in tolerant rats to HAL, animals treated for 10 days with HAL (5 $\mu\text{mol/kg}$) were challenged with these drugs, and the results are shown in Table II. An *i.p.* challenge dose of 5 $\mu\text{mol/kg}$ of CLO failed to change plasma CS levels in HAL-tolerant animals as did HAL challenge. CPZ was reported to affect the pituitary-adrenal axis.⁵⁾ In the present study, an acute *i.p.* administration of 20 $\mu\text{mol/kg}$ of CPZ to non-tolerant rats also elicited a similar increase of plasma CS to that evoked by the acute HAL (5 $\mu\text{mol/kg}$) administration. When a challenge dose of 20 $\mu\text{mol/kg}$ of CPZ was given *i.p.* to HAL-tolerant rats, there was a significant elevation of plasma CS above that of the vehicle control, but its degree of elevation was much smaller than that produced by the acute CPZ treatment in non-tolerant rats. Thus, the chronically HAL-treated rats exhibited a complete cross-tolerance to CLO and a nearly complete cross-tolerance to CPZ.

It was possible that the tolerance to the butyrophenones could have been due to changes in functions of the adrenal cortex. To explore this possibility, rats chronically treated for 10 days with 5 $\mu\text{mol/kg}$ of HAL were injected *s.c.* with 10 U/kg of ACTH 24 h after the last treatment. One hour after the ACTH injection the animals were killed. As shown in Fig. 5, the ACTH injection caused marked rises in plasma CS levels in either non-tolerant or tolerant group of animals. No significant difference in the plasma CS responses to exogenous ACTH between the two groups was observed, indicating that the adrenal cortices of the tolerant animals retained normal responsiveness to ACTH.

DISCUSSION

The results of the present study have clearly demonstrated that chronic treatment of rats with HAL and CLO caused tolerance to the stimulatory

FIG. 5. Adrenocortical Responsiveness to Exogenous ACTH in Rats pretreated chronically with Haloperidol

The animals received a daily *i.p.* injection of haloperidol (5 $\mu\text{mol/kg}$) or vehicle for 10 days. Then they were dosed with haloperidol (5 $\mu\text{mol/kg}$, *i.p.*), ACTH (10 U/kg, *s.c.*) or vehicle (*i.p.*) 24 h after the last treatment and killed by decapitation 1 h later. Data represent the mean $\pm$ S.E. (vertical bars) for 5 to 8 animals.

□, vehicle + vehicle; ▨, vehicle + ACTH; ▤, haloperidol + haloperidol; ■, haloperidol + ACTH.

a) Significantly different at a *p* value of 0.001. NS, not significant.

ry effect of these drugs on the pituitary-adrenal activity. Similar tolerance was also reported with centrally acting agents such as morphine⁶⁾ and diazepam.⁷⁾

There were reports that chronic treatment of rats with a relatively high dose of either HAL or CLO resulted in a decreased activity of hepatic drug-metabolizing enzyme system⁴⁾ by which these drugs were N-dealkylated to pharmacologically inactive metabolites.⁸⁾ Therefore, the chronic treatment may bring about an increase in blood and other tissue levels of HAL and CLO, and hence tolerance may not be due to altered pharmacokinetic mechanisms such as a diminished concentration of the drug at receptor sites responsible for its effects on the pituitary-adrenal system.

Tolerance to HAL is not specific for HAL, because rats tolerant to HAL were also tolerant to CLO and CPZ. Thus, these drugs seem to share the same mechanisms for the drug action on the pituitary-adrenal system.

The adrenocortical response to the butyrophenones is mediated through ACTH release.^{1,2)} Tolerance seems to occur in the ACTH release mechanism of the hypothalamo-pituitary axis and not in the adrenal cortex, since the adrenal cortices of the rats receiving a chronic HAL treatment showed a normal response to exogenous ACTH.

CLO, but not HAL, caused enlargement of the adrenals of rats following chronic treatment as observed in our earlier study with a dose (10 mg/kg) higher than that used in this study.⁴⁾ The enlargement of adrenals may be caused by the direct actions of the drug, because the drug is accumulated in relatively high levels and remain for a long period of time in the adrenals.⁹⁾ Givant *et al.* have also reported that chronic treatment of rats with a high dose (20 mg/kg) of HAL and trifluoperidol resulted in an enlargement of the adrenals,¹⁰⁾ but the mechanisms are yet unclear.

Chronic treatment with HAL failed to raise

the resting level of plasma CS. This result is inconsistent with our earlier work with male rats,⁴⁾ but the discrepancy between these results may be explained by differences in sex of animals, in doses of the drug and/or in routes of administration of the drug.

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