EFFECTS OF NEUROLEPTIC BUTYROPHENONES ON PITUITARY-ADRENAL ACTIVITY IN RATS

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The effects of some neuroleptic butyrophenones such as haloperidol, clofluperol, trifluperidol, lenperone, moperone, and floropipamide on pituitary-adrenal activity were studied in rats following a single *i.p.* administration. All the drugs examined caused a marked increase in the plasma and adrenal corticosterone concentrations. The actions of haloperidol, clofluperol, and trifluperidol were shown to be most potent, while floropipamide was the weakest among them. The stimulatory effect of the drugs on adrenocortical activity was completely inhibited by either dexamethasone pretreatment or hypophysectomy and was shown not to be merely due to a consequence of a drug-induced hypothermia.

These results indicate that the release of ACTH from the adenohipophysis is necessary for the drug action.

Keywords—haloperidol; neuroleptic butyrophenones; pituitary-adrenocortical activity; plasma and adrenal corticosterone; dexamethasone; hypophysectomy; hypothermia

INTRODUCTION

Haloperidol, a butyrophenone, is a potent neuroleptic drug and has been used extensively in clinical practice. This drug is also known to modify secretion of adenohipophysial hormones such as prolactin,¹⁻⁷⁾ growth hormone,^{4,7)} thyroid stimulating hormone,^{4,7)} luteinizing hormone,^{3,8)} follicle stimulating hormone,^{3,8)} and adrenocorticotrophic hormone (ACTH),⁸⁾ resulting in the hyper- or hypo-function of the target glands.^{2,3,9-11)}

A single¹²⁾ or chronic¹³⁾ administration of droperidol (dehydrobenzperidol) was reported to cause hypersecretion of corticosteroid in rats, while a single administration of droperidol¹⁴⁾ or a chronic coadministration of haloperidol and spiroperidol¹⁵⁾ elevated plasma cortisol levels in man. Relatively high doses of haloperidol,¹¹⁾ trifluperidol¹¹⁾ or clofluperol¹⁶⁾ caused enlargement of adrenals of rats, when they were treated chronically. When rats were treated chronically with

haloperidol or clofluperol, an elevation of plasma corticosterone levels was observed.¹⁶⁾ These adrenal responses to the butyrophenones suggest an increase in activity of pituitary-adrenal axis. The effects of these drugs on pituitary-adrenal activity, however, are less clear.

The purpose of this study, therefore, is to investigate the acute effects of some butyrophenones; mainly of haloperidol, on pituitary-adrenal activity in rats by examining the changes in plasma and adrenal corticosterone concentrations.

MATERIALS AND METHODS

Drugs and Chemicals—Haloperidol, clofluperol, and trifluperidol (Dainippon Pharmaceutical Co., Ltd.), floropipamide (Eisai Co., Ltd.), lenperone (Kaken Chemical Co., Ltd.), moperone (Yamanouchi Pharmaceutical Co., Ltd.) were used in this experiment. All butyrophenones used were hydrochlorides. Corticosterone was ob-

tained from Sigma Chemical Co., dexamethasone phosphate (Decadron®) from Banyu Pharmaceutical Co., Ltd. and synthetic ACTH₁₋₂₄ (Cortrosyn®) from Daiichi Pharmaceutical Co., Ltd. All other chemicals were of reagent grade.

Animals—Female Wistar rats (60–80 days old, 140–170 g body weight) were housed at 24±2° on a 12–12 hr light–dark cycle (06:00 light onset) and maintained on a laboratory chow (Oriental Yeast Co., Ltd.) and water *ad libitum*. They were acclimatized to both handling and *i.p.* injection of 0.1 ml of 0.9% saline twice daily for at least 5 consecutive days before the experiments. To avoid influences of daily fluctuations in plasma corticosterone levels, the experiments were carried out between 08:00 and 11:00 with the exception of the time course study which was performed between 08:00 and 18:30.

Time Course and Dose Response—The time course of adrenocortical responsiveness to haloperidol was assessed by killing rats at various times following the *i.p.* administration of 5 µmol/kg of the drug or vehicle at 08:00. In the dose–response study, rats received various doses of the butyrophenones *i.p.* and were sacrificed 1 hr after drug administration.

Site of Drug Action—To determine whether the drug was acting directly on the adrenal cortex, rats were pretreated with 5 mg/kg of dexamethasone *i.p.* 1 hr prior to receiving 5 µmol/kg of haloperidol *i.p.* In another experiment, rats were hypophysectomized by the transauricular approach 24 hr before receiving the butyrophenones (10 µmol/kg, *i.p.*), ACTH (10 U/kg, *s.c.*) or vehicle (*i.p.*). Completeness of hypophysectomy was confirmed by visual inspection at autopsy.

Induction of Hypothermia—Rats received graded doses of haloperidol *i.p.* and their rectal temperatures were determined with an electrothermometer (Natsume Seisakusyo) 0.5 and 1 hr later.

Dissociation from Hypothermia—As cold stress activates the pituitary–adrenal system,¹⁷⁾ it was important to determine whether the effect of haloperidol was merely a secondary consequence of

drug-induced hypothermia. Rats were, therefore, habituated for 10 days to a 38.5° plastic cage which was warmed on a water–bath. On day 11 they were removed briefly for the *i.p.* injection of 10 µmol/kg of the drug, immediately returned to the cage, and then killed 1 hr later. Any significant fall in body temperature of drug-treated animals was not observed in this method (the warmed cage procedure).

Corticosterone Determination—The animals were killed by decapitation within 15 sec after removal from cages. Plasma samples were obtained by collecting trunk blood in heparinized tubes and centrifuging at 1500 × *g* for 15 min at room temperature. Plasma aliquots of 2.0 ml were stored at –20° for subsequent assay. Adrenals, trimmed of adherent fat tissue, were weighed and frozen at –20°. The plasma and adrenal corticosterone contents were determined according to the spectrofluorimetric methods of Zenker and Bernstein,¹⁸⁾ and Givner and Rochefort,¹⁹⁾ respectively.

RESULTS

Time Course of Effect of Haloperidol on Plasma Corticosterone Levels

As shown in Fig. 1, a maximum response of plasma corticosterone to the drug occurred at 1 hr and then the plasma corticosterone decreased to its minimum level at 3 hr after drug administration. On the other hand, the plasma corticosterone in control groups was in low level for up to 3 hr after vehicle treatment. From about 3 hr after treatment, the plasma corticosterone concentrations in both drug-treated and control groups were gradually elevated as reflected by daily rhythms.^{20,21)} However the plasma concentration of corticosterone in drug-treated rats 6 hr after dosing was still significantly higher than that in control rats.

Dose–Response of Plasma and Adrenal Corticosterone to Butyrophenones

The responses of plasma and adrenal corticosterone to haloperidol were examined by killing rats 1 hr after drug treatment, since this was found to be the time of maximum corticosterone response in the time course study. The response of plasma corticosterone to haloperidol was shown to be ap-

FIG. 1. *Time Course of Effect of Haloperidol on Plasma Corticosterone Levels in Rats*

Each point indicates the mean $\pm$ S.E. (vertical bars) for 5 to 13 animals. Female Wistar rats were dosed with haloperidol (5 μ mol/kg) or vehicle i.p. at 08:00 and killed by decapitation at various time intervals after dosing.

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

●—●, control; ○—○, haloperidol.

proximately dose-dependent (Table I). Similar responses of the adrenal corticosterone to haloperidol were also observed (Table I). The responses of plasma and adrenal corticosterone to other butyrophenones such as clofluperol, trifluperidol, lenperone, moperone, and floropipamide are also presented in Table I. Although the extent of responses varied with the drugs tested, all these drugs caused increases in both the plasma and adrenal corticosterone levels as haloperidol did. Haloperidol, clofluperol, and trifluperidol were the most potent drugs examined and floropipamide was the least.

Site of Drug Action

Dexamethasone, a potent inhibitor of ACTH secretion,²²⁾ would be expected to block the corticosterone elevation if the butyrophenones did not directly stimulate the adrenal. As shown in Fig. 2, dexamethasone did not modify any resting plasma corticosterone level, but completely blocked the plasma corticosterone elevation following haloperidol administration. In other experiments,

FIG. 2. *Inhibition of Plasma Corticosterone Response to Haloperidol by Dexamethasone in Rats*

Data are the mean $\pm$ S.E. (vertical bars) for 4 to 6 animals. The animals were dosed with dexamethasone (5 mg/kg) or vehicle i.p. 1 hr before the i.p. administration of haloperidol (5 μ mol/kg) and killed by decapitation 1 hr after the administration of haloperidol.

Open column, water+water; stippled column, water+haloperidol; diagonally hatched column, dexamethasone+water; closed column, dexamethasone+haloperidol.

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

both haloperidol and clofluperol failed to increase the plasma corticosterone in hypophysectomized rats whose adrenal cortices retained the normal responsiveness to exogenous ACTH (Fig. 3). Clofluperol, the most potent neuroleptic among the butyrophenones used, unexpectedly suppressed the plasma corticosterone levels. The suppression by the drug was stronger at 2 hr than at 1 hr after dosing.

Dissociation from Hypothermia

The changes in the rectal temperature following haloperidol injection were examined, because this drug could induce hypothermia as reported by Massotti²³⁾ (Table II). Haloperidol (10 μ mol/kg) produced a decrease of 1.4° in the rectal temperature at 1 hr. To examine whether the plasma corticosterone response is simply a consequence of hypothermia induced by the drug or not, the body

TABLE I. *Effects of Neuroleptic Butyrophenones on Plasma and Adrenal Corticosterone Levels in Rats*

Drug	Dose ($\mu\text{mol/kg}$)	Corticosterone levels	
		Plasma ($\mu\text{g}/100\text{ ml}$)	Adrenal ($\mu\text{g}/10\text{ mg}$)
Control	—	6.9 ± 0.8 (13)	0.17 ± 0.02 (14)
	2	35.0 ± 8.6^a (12)	0.38 ± 0.08^a (12)
Haloperidol	5	96.6 ± 9.3^a (5)	0.42 ± 0.09^a (6)
	10	115.8 ± 2.9^a (4)	0.95 ± 0.03^a (5)
Clofluperol	2	50.3 ± 5.7^a (7)	0.49 ± 0.04^a (8)
	5	102.4 ± 13.1^a (5)	0.73 ± 0.15^a (5)
	10	109.6 ± 4.1^a (8)	0.72 ± 0.03^a (9)
Trifluoperidol	2	47.8 ± 9.2^a (5)	0.39 ± 0.09^a (6)
	5	110.6 ± 4.5^a (5)	0.80 ± 0.03^a (5)
	10	113.9 ± 3.5^a (6)	0.82 ± 0.03^a (6)
Lenperone	2	9.6 ± 1.4 (6)	0.13 ± 0.03 (6)
	5	95.9 ± 5.5^a (5)	0.58 ± 0.06^a (6)
	10	121.1 ± 6.2^a (4)	0.88 ± 0.04^a (6)
Moperone	5	16.8 ± 4.5 (6)	0.14 ± 0.01 (6)
	10	50.1 ± 6.3^a (4)	0.33 ± 0.06^a (6)
Floropipamide	10	10.6 ± 2.5 (6)	0.19 ± 0.04 (6)
	20	39.8 ± 10.0^a (6)	0.32 ± 0.11 (6)

Each value indicates the mean $\pm$ S.E. for the number of animals in parenthesis.

The animals were dosed with the drug or vehicle i.p. at 08:00 and killed by decapitation 1 hr later.

a) Significantly different from control at a p value of 0.05 or less.

TABLE II. *Hypothermia Induced by Haloperidol in Rats*

Dose ($\mu\text{mol/kg}$)	Group	Rectal temperature ($^{\circ}$)		ΔT
		0.5 hr	1 hr	
2	Control	38.7 ± 0.1	38.5 ± 0.1	
	Treated	38.3 ± 0.1	38.1 ± 0.1	$(-0.4)^a$
5	Control	38.9 ± 0.1	39.0 ± 0.1	
	Treated	38.7 ± 0.1	38.6 ± 0.1	$(-0.4)^a$
10	Control	38.4 ± 0.1	38.4 ± 0.1	
	Treated	37.7 ± 0.3	37.0 ± 0.3	$(-1.4)^a$

Each value indicates the mean $\pm$ S.E. for 5 to 6 animals.

The animals were dosed with the drug or vehicle i.p. and their rectal temperatures were determined with an electrothermometer 0.5 and 1 hr after dosing.

a) Significantly different from control at a p value of 0.05 or less.

temperature in drug-treated animals was maintained normally by the warmed cage procedure. As shown in Fig. 4, the pituitary–adrenocortical effect of the drug was shown to be independent of its hypothermic action, since placement of animals in

a 38.5° cage resulted in no attenuation of the plasma corticosterone response. Mean plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml} \pm \text{S.E.}$) were as follows: at $24 \pm 2^{\circ}$ (control, 6.9 ± 0.8 ; drug-treated, 115.8 ± 2.9 , Table I); at 38.5° (control,

FIG. 3. Effects of Haloperidol and Clofluperol on Plasma Corticosterone Levels in Hypophysectomized Rats

Data are the mean $\pm$ S.E. (vertical bars) for 5 to 6 animals. The animals were dosed with the drug (10 μ mol/kg, i.p.), ACTH (10 U/kg, s.c.) or vehicle (i.p.) 24 hr after hypophysectomy.

Open column, control; stippled column, haloperidol; diagonally hatched column, clofluperol; closed column, ACTH.

a) Significantly different from the respective control at a *p* value of 0.05 or less.

6.8 $\pm$ 0.5; drug-treated, 113.2 $\pm$ 6.4).

DISCUSSION

Several reports have shown that droperidol causes increased corticosteroidogenesis in the adrenals of rats,^{12,13)} and that droperidol¹⁴⁾ and some other neuroleptic butyrophenones¹⁵⁾ cause a raise in plasma cortisol levels in man.

The present study with some butyrophenones such as haloperidol, clofluperol, trifluperidol, lenperone, moperone, and floropipamide showed the elevation of plasma corticosterone in rats. The strength of the stimulatory effect was decreased in the order of haloperidol, clofluperol, and trifluperidol > lenperone > moperone >

FIG. 4 Effect of Blocking Haloperidol-induced Hypothermia on Plasma Corticosterone Response

Data are the mean $\pm$ S.E. (vertical bars) for 5 animals. The animals were habituated to a 38.5° cage for 10 days prior to the experiment. Then they were dosed with the drug (10 μ mol/kg) or vehicle i.p. and killed by decapitation 1 hr later.

Open column, control; stippled column, haloperidol.

a) Significantly different from control at a *p* value of 0.001.

floropipamide. The order seems to approximately correlate with their pharmacological activities.²⁴⁾ The stimulation of adrenocortical activity is completely inhibited by either dexamethasone pretreatment or hypophysectomy, indicating that the site of drug action is not the adrenal cortex and that the effect of the drugs does require ACTH secretion from the adenohypophysis. Our study also shows that the effect is not a secondary consequence of a drug-induced fall in body temperature.

Haloperidol¹⁻⁷⁾ and melperone²⁵⁾ have also been known as potent stimulators of prolactin release from the pituitary. Several lines of evidence have indicated that prolactin inhibits adrenal 5 α -reductase activity followed by decreasing intra-adrenal metabolism of corticosterone.²⁶⁻²⁸⁾ The metabolic consequence leads to increase in the output of corticosterone from the adrenals. The increased output of corticosterone by prolactin is much less than by ACTH, since prolactin, unlike

ACTH, does not stimulate steroidogenesis *per se* with increased production of corticosterone from precursor.²⁸⁾ But the elevation of the plasma and adrenal corticosterone observed in this study seems to result, at least in part, from the action on the adrenals of prolactin released by haloperidol and other butyrophenones used. The mechanisms by which the drugs activate the pituitary-adrenocortical system are still unknown and are under investigation.

Besides stimulatory effects, clofluperol suppresses the plasma corticosterone levels in hypophysectomized rats. In our previous reports, the drug has shown to interfere with the structure of biological membrane^{29,30)} and to have a high affinity for the adrenals.³¹⁾ Thus, it is possible that the drug may interact nonspecifically with the adrenocortical cell membrane and the resulting membrane stabilization may cause decreased secretion of the corticosteroids. In intact animals, the effect of ACTH released by the drug seems likely to precede or overcome direct action of the drug on the adrenals. But the detailed mechanisms by which the drug acts on the adrenals are not yet clarified.

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