A Kinetic Study of Chlorpromazine on the Hyperglycemic Response in Rats. I. Effect of Chlorpromazine on Plasma Catecholamines

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The effect of chlorpromazine (0.5 and 4 mg/kg) on plasma catecholamines (adrenaline and noradrenaline) concentrations was investigated in rats. After an i.v. bolus administration of chlorpromazine, plasma adrenaline and noradrenaline concentrations showed a dose dependent increase. In order to clarify the pharmacokinetics of catecholamines in plasma, i.v. infusion of adrenaline or noradrenaline was also carried out. Plasma catecholamines after i.v. infusion showed typical characteristics of a one compartment model with a zero order production rate of endogenous catecholamines. From the data observed, a mathematical model was constructed to elucidate the relationship between the pharmacokinetics (brain concentrations) and the pharmacologic effect (plasma catecholamines concentrations) of chlorpromazine in rats. The results indicated that the time courses of plasma adrenaline and noradrenaline concentrations after an i.v. administration of chlorpromazine were described reasonably well by a simple pharmacokinetic-pharmacodynamic model.

Keywords — chlorpromazine; hyperglycemia; pharmacokinetics; pharmacodynamics; adrenaline; noradrenaline

Introduction

Many reports have indicated that chlorpromazine (CPZ) induces hyperglycemia in several species.¹⁾ Since the hyperglycemic effect is inhibited by adrenalectomy, adrenal demedulation and by pretreatment with ganglionic blocking agents, it has been generally agreed that the hyperglycemia due to CPZ is closely related to the release of catecholamines from the adrenal medulla.²⁾ Fujimori *et al.*³⁾ demonstrated that CPZ stimulated the central nervous system to release adrenaline (ADR) from the adrenal medullas of rats. It was also reported that CPZ increased the urinary excretion of catecholamines *in vivo*.⁴⁾ Although there are many reports on the mechanisms of the hyperglycemic, hypothermic and neuroleptic effects of CPZ, there have been only a few attempts to investigate the relationship between pharmacokinetics and pharmacodynamics of CPZ *in vivo*.

In the present study, the time courses of plasma concentrations of catecholamines after intravenous administration of CPZ were deter-

mined to study the pharmacologic effect of CPZ. The purpose of this study was to clarify the concentration-effect relationship of CPZ in the rat, using a mathematical model.

Materials and Methods

Chemicals — Chlorpromazine hydrochloride (CPZ, JP grade, Nakarai Chemical Co., Kyoto, Japan), *l*-adrenaline (ADR, Bosmin® Inj., JP grade, Daiichi Seiyaku Co., Tokyo, Japan) and *l*-noradrenaline (NOR, reagent grade, Nakarai Chemical Co., Kyoto) were purchased commercially and were used without further purification. All other chemicals were of reagent grade and were also obtained commercially.

Animal Experiments — Male albino rats (Wistar strain, Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing 250 to 350 g were used. Under light ether anesthesia, each rat was cannulated with a polyethylene tubing (PE50, Clay Adams, Parsippany, NJ) which was attached with a Silastic medical grade

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tubing (0.51 mm in i.d. and 0.94 mm in o.d., Dow Corning Co., Midland, MI) into the left femoral vein (for catecholamines infusion) and the right jugular vein (for drug administration and blood sampling). Catheters were tunneled under the skin to exit at the nape of the neck. All animals were allowed at least an overnight period for recovery, with water and food available. CPZ was dissolved in 1 ml of physiological saline (JP XI, Otsuka Pharmaceutical Co., Tokyo, Japan) and was administered into the jugular vein in dose levels of 0.5 and 4 mg/kg. Blood samples (0.45 ml each) were withdrawn from the jugular vein at 10, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after the administration. The blood samples were mixed with 10 μ l of pH 7.0 buffer solution containing ethyleneglycol bis(β -aminoethylether) *N, N, N', N'*-tetraacetic acid (EGTA; 90 mg/ml) and glutathione (60 mg/ml), immediately. After centrifugation (at 10000 rpm for 2 min), 200 μ l of plasma were obtained and stored at -20°C until analysis. ADR or NOR was dissolved in physiological saline and was infused *via* the

femoral vein at a rate of 40 $\mu\text{g/kg/h}$ (flow rate: 0.032 ml/min) for 30 min using an infusion pump (KN type, Natume Seisakusho Co., Tokyo, Japan). Blood samples (0.45 ml) were withdrawn from the jugular vein and were treated in the same manner as described above. All animal experiments were carried out in the conscious and unrestrained condition at the room temperature of $23 \pm 1^{\circ}\text{C}$ with the relative humidity of 50%. Every experiment was started at 11 a.m. to minimize the diurnal variation in rats.

Analytical Method of Catecholamines — Plasma catecholamines were determined by a radioenzymatic method (CAT-A-KIT, Upjohn Co., Kalamazoo, MI).

Estimation of model parameters was carried out as described previously.^{5a)}

Results and Discussion

Effect of CPZ on Plasma Catecholamines

Time courses of plasma ADR and NOR levels before and after i.v. administration of

Fig. 1. Time Course of Plasma ADR Levels after an Intravenous Administration of CPZ

Upper panel; 0.5 mg/kg, lower panel; 4 mg/kg. The plotted points represent the observed data and the solid lines represent the calculated values using Eq. 5 in the text. CPZ was administered at time zero and the ADR values at time zero represent the control levels. Each experimental point is shown as the mean $\pm$ S.E. ($n = 3$).

Fig. 2. Time Course of Plasma NOR Levels after an Intravenous Administration of CPZ

Upper panel; 0.5 mg/kg, lower panel; 4 mg/kg. The plotted points represent the observed data and the solid lines represent the calculated values using Eq. 6 in the text. CPZ was administered at time zero and the NOR values at time zero represent the control levels. Each experimental point is shown as the mean $\pm$ S.E. ($n = 3$).

CPZ (0.5 and 4 mg/kg) are shown in Figs. 1 and 2, respectively. The control levels of plasma ADR and NOR were 0.056 and 0.168 ng/ml, respectively, and these values are consistent with the report of Micalizzi *et al.*⁶⁾ After CPZ administration, the plasma ADR and NOR concentrations were increased in a dose dependent manner. At 4 mg/kg dose, the maximum plasma ADR concentration was about 0.23 ng/ml at 20 min after dosing and gradually returned to the control level thereafter. While, the maximum level in plasma NOR concentration was about 1.4 ng/ml at 10 min after CPZ injection which also returned gradually to the control level.

Several investigators have suggested that the hyperglycemic effect of CPZ is closely related to the sympathetic nerve stimulation and ADR release from the adrenal medulla.²⁾ The present results indicates consistency with the literature.

Infusion of Catecholamines

The time courses of plasma ADR and NOR levels before, during and after constant rate i.v. infusion of catecholamines (40 μ g/kg/h for 0.5 h) are shown in Fig. 3 (ADR) and Fig. 4

(NOR). Both catecholamines levels reached peak concentrations just after the start of infusion, suggesting that ADR and NOR had extremely short half lives in plasma.

Description of the Model

In order to clarify the relationship between CPZ disposition and plasma catecholamine concentration, a kinetic model was constructed with the following assumptions, (1) the dispositions of both catecholamines, ADR and NOR, can be described by one-compartment open model; (2) the endogenous catecholamines are produced by zero order rate kinetics and are eliminated from plasma by first order kinetics; (3) the site of action of CPZ is in the brain and (4) the production rates of ADR and NOR increase in proportion to the brain (biophase) concentration of CPZ directly. A schematic diagram of the model is shown in Fig. 5. After an intravenous infusion of either ADR or NOR, the change in plasma catecholamines were described by the following equations.

$$\frac{dC_{\text{ADR}}}{dt} = k_{\text{ADR}0} + \frac{I_{\text{ADR}}}{V_{\text{d,ADR}}} - k_{\text{ADR}e} C_{\text{ADR}} \quad (1)$$

Fig. 3. Time Course of Plasma ADR Levels before, during and after the Constant Infusion of ADR in Rats

The plotted points represent the observed data and the solid line represents the calculated values using Eq. 1 in the text. Each experimental point is shown as the mean $\pm$ S.E. ($n = 3$).

Fig. 4. Time Course of Plasma NOR Levels before, during and after the Constant Infusion of NOR in Rats

The plotted points represent the observed data and the solid line represents the calculated values using Eq. 2 in the text. Each experimental point is shown as the mean $\pm$ S.E. ($n = 3$).

Fig. 5. Diagrammatic Representation of the Effect of CPZ on the Plasma Catecholamines in the Rat

$$\frac{dC_{\text{NOR}}}{dt} = k_{\text{NOR}0} + \frac{I_{\text{NOR}}}{V_{\text{d,NOR}}} - k_{\text{NORE}} C_{\text{NOR}} \quad (2)$$

where C_{ADR} and C_{NOR} are the plasma concentrations of ADR and NOR, $k_{\text{ADR}0}$ and $k_{\text{NOR}0}$ are the production rates of endogenous ADR and NOR, I_{ADR} and I_{NOR} are the infusion rates of ADR and NOR, $V_{\text{d,ADR}}$ and $V_{\text{d,NOR}}$ are the distribution volume of ADR and NOR, and k_{ADRe} and k_{NORE} are the first-order rate constants for the elimination of ADR and NOR, respectively. Before the infusion, Eqs. 1 and 2 are reduced to Eqs. 3 and 4, respectively.

$$0 = k_{\text{ADR}0} - k_{\text{ADRe}} C_{\text{ADR}0} \quad (3)$$

$$0 = k_{\text{NOR}0} - k_{\text{NORE}} C_{\text{NOR}0} \quad (4)$$

where $C_{\text{ADR}0}$ and $C_{\text{NOR}0}$ are the plasma concentrations of ADR and NOR, respectively at the control state (before medication). The endogenous catecholamine production rates, $k_{\text{ADR}0}$ and $k_{\text{NOR}0}$, were calculated by these equations and are listed in Table I.

The plasma catecholamine concentrations after CPZ administration were described by the following equations.

TABLE I. Pharmacokinetic and Pharmacodynamic Parameters

$k_{\text{ADR}0}$	ng/ml/h	2.132
k_{ADRe}	h^{-1}	38.03
$V_{\text{d,ADR}}$	ml/kg	813.6
$k_{\text{NOR}0}$	ng/ml/h	6.903
k_{NORE}	h^{-1}	41.09
$V_{\text{d,NOR}}$	ml/kg	949.7
α	ng/ml/h/ ($\mu\text{g}/\text{ml}$)	0.541
ν	ng/ml/h/ ($\mu\text{g}/\text{ml}$)	3.363

$$\frac{dC_{\text{ADR}}}{dt} = k_{\text{ADR}0} + \alpha C_{\text{BR}} - k_{\text{ADRe}} C_{\text{ADR}} \quad (5)$$

$$\frac{dC_{\text{NOR}}}{dt} = k_{\text{NOR}0} + \nu C_{\text{BR}} - k_{\text{NORE}} C_{\text{NOR}} \quad (6)$$

where C_{BR} is the brain concentration of CPZ, and α and ν are the proportional constants for the pharmacologic response of CPZ on the production rates of endogenous ADR and NOR, respectively.

Data Fitting and Estimation of the Model Parameters

The solid lines shown in Figs. 3 and 4 represent the calculated values of the time courses of ADR and NOR, according to Eqs. 1 and 2, respectively. Although the model employed here was relatively simple, the calculated values adequately described the observed data. The estimated parameters are shown in Table I. Among the parameters, the products of $k_{\text{ADR}0}$ and V_{ADR} or $k_{\text{NOR}0}$ and V_{NOR} represent the endogenous production rate of the catecholamine in plasma at the control state. The present result is fundamentally consistent with the result of Robinson *et al.*^{7a)} in dogs, on a body weight basis.

In order to clarify the elimination kinetics of catecholamines in plasma, we used relatively high infusion rates of exogenous catecholamines. Since there is a homeostatic system for the blood pressure by the adrenal, plasma catecholamine levels during and after a small amount of catecholamine infusion might be influenced considerably by the change of endogenous catecholamine productions in the body. Thus, relatively high dosages of catecholamines

were indispensable for obtaining the disposition constants of catecholamines. In the present study, the elimination kinetics of ADR and NOR from plasma were assumed to be simple first order kinetics without any control systems. It is well known that the metabolic pathways of catecholamines are O-methylation, deamination and conjugation. Since there has been no report indicating that these metabolic pathways are controlled by the blood pressure homeostatic system in the body, a simple first order model was used in this study. As shown in Table I, the elimination half lives for ADR and NOR were about 1 min, and this result is consistent with the report of Axelrod *et al.*^{7b)}

The solid lines shown in Figs. 1 and 2 are the calculated values of plasma ADR and NOR levels after i.v. administration of CPZ, according to Eqs. 1 through 6. The values for C_{BR} in Eqs. 5 and 6 were calculated by a blood flow limited model, according to the previous paper, and all of the pharmacokinetic parameters for CPZ disposition were adopted from the previous report.^{5a)} The calculated values for C_{ADR} and C_{NOR} agree pretty well with the observed data. Pharmacodynamic parameters used in the calculation are also listed in Table I.

In order to describe the relationship between pharmacologic intensity and the concentration of the drug quantitatively, many types of models such as the linear model, the log-linear model, the E_{max} model and the sigmoid E_{max} model (Hill's equation) have been suggested.⁸⁾ In previous papers, we reported that the effect of CPZ on the body temperature was correlated with the brain level of CPZ, using a conventional Hill's equation and a body temperature-regulation model.⁵⁾ Accordingly, we calculated the effect of CPZ on the plasma catecholamines using a Hill's equation, as shown in the following equations.

$$\frac{dC_{ADR}}{dt} = k_{ADR0} + \frac{E_{max,ADR} C_{BR}^{r1}}{EC(50)_{ADR}^{r1} + C_{BR}^{r1}} - k_{ADRe} C_{ADR} \quad (7)$$

$$\frac{dC_{NOR}}{dt} = k_{NOR0} + \frac{E_{max,NOR} C_{BR}^{r2}}{EC(50)_{NOR}^{r2} + C_{BR}^{r2}} - k_{NORe} C_{NOR} \quad (8)$$

where $E_{max,ADR}$ and $E_{max,NOR}$ are the maximum effects of CPZ, $EC(50)_{ADR}$ and $EC(50)_{NOR}$ are the CPZ concentrations which produce 50% of E_{max} values, and $r1$ and $r2$ are the Hill's constants of ADR and NOR, respectively. The calculated curves of ADR and NOR after i.v. administration of CPZ also agreed with the corresponding observed data using the following pharmacodynamic parameters; $r1 = 0.821$, $EC(50)_{ADR} = 4.98$ mg/ml, $E_{max,ADR} = 0.899$ mg/ml/h, $r2 = 0.849$, $EC(50)_{NOR} = 2.54$ mg/ml and $E_{max,NOR} = 3.73$ mg/ml/h. However, some of the parameters such as $EC(50)_{ADR}$ and $EC(50)_{NOR}$ were extremely large compared with the pharmacologic significance. Data analysis using the Akaike's information criterion (AIC)⁹⁾ was also carried out. In the linear model (Eqs. 5 and 6), AIC values of the ADR and NOR studies were -77.8 and -17.6 , respectively. Whereas, in the sigmoid E_{max} model (Eqs. 7 and 8), AIC values of the ADR and NOR studies were -74.1 and -10.7 , respectively. These results suggest that the linear model, rather than the sigmoid E_{max} model, is more suitable for the description of the effect of CPZ on the secretion of catecholamines in rats. Therefore we adopted a linear function in this study. The effect of CPZ on the plasma catecholamine levels were reasonably described by the linear model. The assumptions described above therefore, might be appropriate.

Pharmacodynamics in respect to plasma catecholamines and pharmacokinetics of CPZ have been investigated. The time course of the pharmacologic effect of CPZ was well explained by a simple pharmacokinetic-pharmacodynamic model. It has been generally agreed that the hyperglycemic effect of CPZ is closely related to the levels of endogenous catecholamines in the body. The relationship between CPZ disposition and the hyperglycemic effect in rats will be elucidated in the subsequent paper.

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