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EFFECT OF CHLORPROMAZINE ON PLASMA ADENOSINE 3′,5′-CYCLIC PHOSPHATE LEVEL

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Abstract—A subcutaneous injection of chlorpromazine hydrochloride (CPZ) at a dose of 10 mg/kg caused an increase in the plasma cyclic AMP level in male mice. Propranolol (2 mg/kg) and hexamethonium (50 mg/kg) abolished the elevation of plasma cyclic AMP induced by CPZ. Phentolamine (5 mg/kg) could not inhibit the effect of CPZ. Adrenalectomy completely inhibited the elevation of plasma cyclic AMP produced by CPZ. Pretreatment with 6-hydroxydopamine (100 mg/kg, i.v., 24 hr before) failed to reduce the elevation of the plasma cyclic AMP level produced by CPZ. Intracerebroventricular administration of CPZ (5–25 μg/mouse) also increased plasma cyclic AMP levels. These findings indicate that CPZ activated the sympathetic nervous system by acting on the CNS, thereby increasing plasma cyclic AMP levels through the stimulation of β-adrenoceptors mainly by catecholamines released from the adrenal medulla.

Recently, the usefulness of plasma cyclic AMP (adenosine 3′,5′-cyclic phosphate) as an *in vivo* indicator for endogenous adrenergic activity was shown by Kunitada *et al.* [1], because generation of the "functioning" cyclic AMP in a particular intracellular site would be reflected in an increase of plasma cyclic AMP, rather than in an increase of tissue content. We have demonstrated previously that the administration of morphine increased plasma cyclic AMP in mice through the activation of the central–adrenal axis [2]. Chlorpromazine is known as a potent blocker of both adrenergic α-receptors and dopaminergic receptors [3, 4]. Recently, the presence of pre-synaptic adrenergic α-receptors has been demonstrated, and differences between pre-synaptic and post-synaptic α-receptors have been discussed [5, 6]. Moreover, several lines of evidence have indicated the differences between central and peripheral adrenergic α-receptors [7]. For example, clonidine is known as a preferential stimulant of the central adrenergic α-receptor, and chlorpromazine is known as its preferential blocker [8]. It is, therefore, probable that chlorpromazine may affect peripheral adrenergic activity by blocking the central and peripheral α-adrenergic receptors. Bonaccorsi *et al* [9] reported that hyperglycemia in rats, induced by intraperitoneally injected chlorpromazine, was associated with an activation of the adrenergic mechanism. Nevertheless, there are no reports as to whether this drug alters the plasma concentrations of catecholamines and/or cyclic AMP.

To know the effect of chlorpromazine on adrenergic activity, we have examined the alteration of plasma cyclic AMP levels after administration of chlorpromazine in mice.

MATERIALS AND METHODS

Male ddY mice weighing 20–30 g were used. The mice were usually injected subcutaneously with chlorpromazine hydrochloride (CPZ) in a dose of 10 mg/kg and were decapitated 30 min later. Blood specimens (0.05 ml each) from trunk blood were mixed with 0.15 ml of saline containing 10 mM EDTA (pH 7.4). After centrifugation, the supernatant fraction was directly analyzed for cyclic AMP by means of the radioimmunoassay microprocedure developed by Honma *et al.* [10].

Adrenalectomy was done through the dorsal approach under pentobarbital anesthesia 24 hr before decapitation, and 0.33 mg/kg of dexamethasone was administrated s.c. at the end of the operation to one group of mice to supplement for the corticosteroid deficit. Sham-operated mice were used as controls. Mice were injected intravenously with 100 mg/kg of 6-hydroxydopamine hydrobromide dissolved in 0.3 M ascorbic acid solution 24 hr before the CPZ administration. Rectal body temperature was recorded with an electric thermometer (Nippon Koden, model MGA-III, Tokyo, Japan). Intracerebroventricular administration of CPZ was done after the method of Haley and McCormick [11], as described by Muraki *et al.* [2].

Sources of reagents were as follows: CPZ, Shionogi Pharmaceutical Co., Osaka, Japan; hexamethonium bromide, Yamanouchi Pharmaceutical Co., Tokyo; 6-hydroxydopamine hydrobromide (6-OHDA) and propranolol hydrochloride, Sigma Chemical Co., St. Louis, MO, U.S.A.; phentolamine mesylate, Japan Chiba Geigy, Takarazuka, Japan; dexamethasone sodium phosphate, Japan Merck Banyu Co., Tokyo, Japan. The cyclic AMP assay kit was obtained from the Yamasa Shoyu Co., Chyoshi, Japan. Concentrations of the drug solutions were prepared to allow an administration volume of 10 ml/kg. Doses were calculated on the basis of the salt of each drug. Other reagents were analytical grade from commercial sources.

All samples from each experiment were measured within a single assay. Results were evaluated using Student's *t*-test.

RESULTS

A subcutaneous injection of CPZ in a dose of 10 mg/kg body weight caused an increase in plasma cyclic AMP, as shown in Fig. 1. Plasma cyclic AMP reached a peak 30 min after the injection of CPZ and returned toward the basal level within 60–90 min. Thirty minutes after administration of CPZ, the increase in plasma cyclic AMP levels was dose-related up to 40 mg/kg, s.c. (Fig. 2). Therefore, in the following experiments the plasma cyclic AMP level was determined 30 min after 10 mg/kg CPZ to investigate the mechanism of the elevation of plasma cyclic AMP induced by CPZ.

To determine if CPZ can produce an elevation of plasma cyclic AMP by increasing adrenergic activity, the effects of various blockers of autonomic nervous system function on the action of CPZ were examined. Table 1 shows the effects of phentolamine, propranolol or hexamethonium treatment on the elevation of plasma cyclic AMP levels produced by CPZ. Pretreatment with propranolol or hexamethonium lowered both the basal levels and the CPZ-induced increase in plasma cyclic AMP, while pretreatment with phentolamine increased both the basal and the elevated cyclic AMP levels.

To determine the role of the adrenal medulla in the action of CPZ, the effect of adrenalectomy on the increase in the plasma cyclic AMP level induced by CPZ was investigated. As shown in Table 2, adrenalectomy completely inhibited the elevation of plasma AMP due to CPZ without affecting the basal level. Dexamethasone did not alter the inhibitory effect of adrenalectomy.

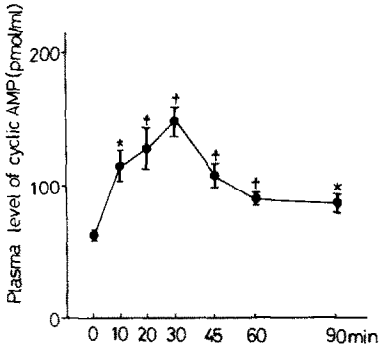

Fig. 1. Time course of the effect of CPZ on the plasma cyclic AMP level. CPZ (10 mg/kg) was injected s.c. at time 0. The columns and vertical bars represent means and standard errors of five mice. Key: *P < 0.05, †P < 0.01 vs the value at 0 min.

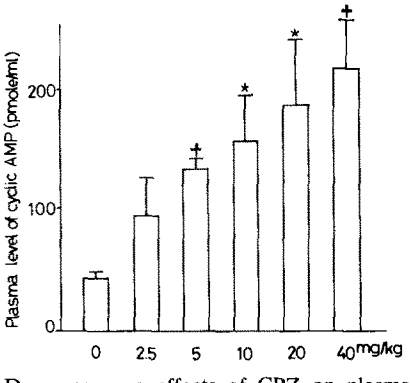

Fig. 2. Dose–response effects of CPZ on plasma cyclic AMP levels. Mice were killed 30 min after subcutaneous administration of CPZ. The columns and vertical bars represent means and standard errors of three mice. Key: *P < 0.05, †P < 0.01 compared to the 0 mg/kg dose.

Table 1. Effect of phentolamine, propranolol or hexamethonium treatment on elevation of plasma cyclic AMP level induced by CPZ*

Expt.	Treatment	Dose (mg/kg)	Plasma level of cyclic AMP (pmoles/ml)
I	Saline		58.7 ± 10.1
	CPZ	10	131.7 ± 8.4†
	Phentolamine	5	98.8 ± 13.1‡
	Phentolamine	5	
	+CPZ	10	191.6 ± 22.6§
II	Saline		50.4 ± 2.7
	CPZ	10	138.4 ± 20.9†
	Propranolol	2	35.7 ± 1.7†
	Propranolol	2	
	+CPZ	10	38.9 ± 5.8‖
	Hexamethonium	50	14.5 ± 1.7†
	Hexamethonium	50	
	+CPZ	10	8.1 ± 2.3‖

* Phentolamine (s.c.), propranolol (i.p.) and hexamethonium (i.p.) were given 5 min before CPZ (s.c.) administration. The plasma cyclic AMP level was determined 30 min after CPZ. Values are means (N = 5) ± standard errors.

† $P < 0.01$ vs saline.

‡ $P < 0.05$ vs saline.

§ $P < 0.05$ vs CPZ and $P < 0.01$ vs phentolamine.

‖ $P < 0.01$ vs CPZ.

The systemic administration of 6-OHDA depleted the catecholamine content in the peripheral catecholaminergic nerve terminals, but not that in the adrenal medulla [2, 12]. To investigate the contribution of the release of catecholamines from the peripheral catecholaminergic nerve terminals to the cyclic AMP effect of CPZ, we examined whether pretreatment with 6-OHDA could lower the increase in cyclic AMP levels elicited by CPZ. In the vehicle-treated mice, the mean plasma cyclic AMP levels, determined 30 min after administration of saline or CPZ, were 55.2 ± 4.8 pmoles/ml or 133.2 ± 15.9 pmoles/ml, while in the 6-OHDA-treated mice, those of saline or CPZ were 79.2 ± 10.3 pmoles/ml or 209.9 ± 27.0 pmoles/ml respectively. Depletion of catecholamines in the nerve terminals by 6-OHDA failed to reduce the elevation of plasma cyclic AMP induced by CPZ, suggesting that the release of catecholamines from catecholaminergic nerve terminals plays a less important role in the elevation of the plasma cyclic AMP induced by CPZ.

The origin of plasma cyclic AMP is yet to be determined; however, liver, muscle and adipose tissue are considered as candidates for the origin of cyclic AMP released by exogenous catecholamines [13, 14]. While searching for the possible origin of plasma cyclic AMP, we investigated whether CPZ increases the hepatic cyclic AMP content. Fifteen minutes after administration of 10 mg/kg CPZ, s.c., the cyclic AMP content of liver (1001 ± 55 pmoles/g tissue) was significantly higher than that of the saline

Table 2. Effect of CPZ on plasma level of cyclic AMP in adrenalectomized mice*

Expt.	Mice	Treatment	Plasma level of cyclic AMP (pmoles/ml)
I	Intact	Saline	69.0 ± 6.0
	Intact	CPZ	159.2 ± 16.4†
	Sham-operated	Saline	65.9 ± 7.7
	Sham-operated	CPZ	152.4 ± 23.9‡
	Adrenalectomized	Saline	60.4 ± 2.8
	Adrenalectomized	CPZ	72.2 ± 2.0
II	Intact	Saline	82.9 ± 5.6
	Intact	CPZ	167.1 ± 32.2§
	Sham-operated	Saline	83.6 ± 7.2
	Sham-operated	CPZ	157.8 ± 10.2‡
	Adrenalectomized + dexamethasone	Saline	70.0 ± 7.7
	Adrenalectomized + dexamethasone	CPZ	76.5 ± 4.6

* CPZ (10 mg/kg, s.c.) was given 24 hr after adrenalectomy with (Expt. II) or without (Expt. I) supplemented dexamethasone (0.33 mg/kg, s.c.). The plasma cyclic AMP level was determined 30 min after CPZ administration. Values are means (N = 5) ± standard errors.
† $P < 0.01$ vs intact, saline.
‡ $P < 0.01$ vs sham-operated, saline.
§ $P < 0.05$ vs intact, saline.

control mice (801 ± 58 pmoles/g tissue). This indicates that the plasma cyclic AMP rise elicited by CPZ was accompanied by an increase in the liver cyclic AMP content.

The intracerebroventricular injection of CPZ (up to 25 μg/mouse) also caused a dose-related increase in plasma cyclic AMP (Fig. 3), although the injection of saline alone caused an increase in the plasma cyclic AMP of 15 per cent. Pretreatment with pentobarbital (60 mg/kg, i.p.) prevented the elevation of plasma cyclic AMP induced by CPZ (data not shown). These findings indicate that the site of action of CPZ was probably the CNS.

Since it is known that CPZ produces hypothermia [9], and hypothermia may increase plasma cyclic AMP levels, there is a possibility that CPZ increases plasma cyclic AMP levels by decreasing body temperature. To exclude this possibility, we examined the effects of CPZ at room temperatures (28 and 33°) at which CPZ did not decrease the body temperature (Table 3). CPZ, at a dose of 10 mg/kg, decreased body temperature by 2 to 3.5° at a room temperature of 22–23°, while it did not at 28 and 33°; CPZ increased the plasma concentration of cyclic AMP significantly, regardless of whether or not hypothermia occurred.

Table 3. Effect of room temperature on CPZ-induced elevation of plasma cyclic AMP levels in mice*

No. of mice	Room temperature (°)	Treatment	Change in body temperature (°)	Plasma level of cyclic AMP (pmoles/ml)
Expt. I				
5	22–23	Saline	−0.18 ± 0.17	80.6 ± 4.7
5	22–23	CPZ	−3.44 ± 0.42†	173.2 ± 23.0‡
5	28	Saline	−0.08 ± 0.15	70.4 ± 4.9
5	28	CPZ	−0.82 ± 0.39§	166.2 ± 10.8‖
Expt. II				
5	22–23	Saline	+0.04 ± 0.35	65.6 ± 2.9
5	22–23	CPZ	−2.28 ± 0.41†	158.4 ± 10.8‡
6	33	Saline	+0.04 ± 0.26	58.0 ± 6.5
6	33	CPZ	+0.50 ± 0.16¶	146.3 ± 11.6**

* The plasma cyclic AMP level and body temperature were determined 30 min after CPZ (10 mg/kg, s.c.). Values are means ± standard errors.
† $P < 0.01$ vs 22–23°, saline.
‡ $P < 0.01$ vs 22–23°, saline.
§ Not significant vs 28°, saline.
‖ $P < 0.01$ vs 28°, saline.
¶ Not significant vs 33°, saline.
** $P < 0.01$ vs 33°, saline.

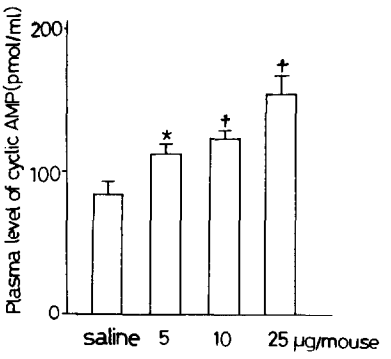

Fig. 3. Effect of intracerebroventricular administration of CPZ on the plasma of cyclic AMP. Cyclic AMP was determined 30 min after the injection of 20 μl of CPZ solution. The columns and vertical bars represent means and standard errors of six mice. Key: *P < 0.05, †P < 0.01 compared to saline-injected mice.

DISCUSSION

It has been shown that an injection of CPZ in rats will cause an elevation in the plasma level of glucose, and that this effect is associated with activation of an adrenergic mechanism [9]. Nevertheless, it is not known whether CPZ actually activates the adrenergic nervous system and increases the plasma catecholamine content.

It has been established that catecholamines increase plasma cyclic AMP levels *in vivo*, and that this effect is inhibited by a β-adrenergic blocking agent [1, 13]. It was shown that plasma cyclic AMP levels were increased by releasing endogenous catecholamines; administration of tyramine caused an increase in plasma cyclic AMP in rats by releasing norepinephrine from the catecholaminergic nerve terminals, while the induction of ether anesthesia increased plasma cyclic AMP by releasing catecholamines from the adrenal medulla [1].

In the present study it was shown that CPZ increases plasma cyclic AMP levels in mice, perhaps as a result of the stimulation of adrenergic activity (Figs. 1 and 2). In order to test this hypothesis, the mechanism of elevation of plasma cyclic AMP induced by CPZ was examined through the effects of adrenolytic agents and adrenalectomy. Pretreatment with propranolol, a specific β-adrenergic blocker, inhibited the elevation of plasma cyclic AMP induced by CPZ (Table 1). Hexamethonium, a ganglionic blocker, also inhibited the increase of the plasma cyclic AMP level induced by CPZ (Table 1). The plasma cyclic AMP level was not elevated by CPZ in the adrenalectomized mice in spite of a supplement of dexamethasone (Table 2). Therefore, depletion of corticosteroids has no causal relationship to the inhibition of the CPZ effect by adrenalectomy. These results, therefore, suggest that CPZ stimulated the release of catecholamines from the adrenal medulla which, in turn, activated tissue adenylate cyclase by stimulating the β-adrenoceptors, and the cyclic AMP thus formed was released into the extracellular space and led to increases in the plasma cyclic AMP levels. The increase in plasma cyclic AMP by CPZ was accompanied by a slight but significant increase in hepatic cyclic AMP content. This suggests that the liver is one of the origins of the plasma cyclic AMP released by CPZ injection.

Since CPZ is known to inhibit phosphodiesterase *in vitro* [15], there is a possibility that CPZ raises the plasma cyclic AMP level by decreasing the phosphodiesterase activity. However, the elevation of the plasma cyclic AMP level produced by CPZ was completely abolished by adrenalectomy, and the intraventricular injection of a small amount of CPZ, up to 25 μg, increased plasma cyclic AMP. These results, therefore, indicated that *in vivo* inhibition of phosphodiesterase activity by CPZ in the dose used may be insufficient to cause an elevation of the plasma cyclic AMP level. Indeed, we observed that even 50 mg/kg (i.p.) of theophylline increased the plasma cyclic AMP level only slightly (data not shown). In addition, complete blockade by propranolol or hexamethonium of the elevation of plasma cyclic AMP induced by CPZ supports our interpretation.

It has been reported that intravenous injection of 6-OHDA destroys the catecholaminergic nerve terminals without affecting the adrenal medulla [2, 12]. In the present experiment, the destruction of catecholaminergic nerve terminals by 6-OHDA could not inhibit the effect of CPZ to increase the plasma cyclic AMP level. Kunitada *et al.* [1] indicated that pretreatment with intravenous 6-OHDA abolished the increase in the plasma cyclic AMP level induced by the release of norepinephrine from the catecholaminergic nerve terminals by tyramine, while it did not inhibit the increase in the plasma cyclic AMP induced by adrenal catecholamines released by hypoglycemia. The failure of inhibition of the CPZ action by 6-OHDA would indicate that norepinephrine from the catecholaminergic nerve terminals plays a less important role than catecholamines from the adrenal medulla in the effect of CPZ on the elevation of plasma cyclic AMP levels.

Though intraventricular injection of saline tended to increase plasma cyclic AMP levels, intraventricular administration of CPZ caused a further increase in the plasma cyclic AMP level. The pretreatment with pentobarbital prevented the elevation of plasma cyclic AMP induced by CPZ. These results suggest that the site of action of CPZ to increase plasma cyclic AMP levels exists in the CNS.

We confirmed the previous finding that phentolamine increases the basal plasma cyclic AMP level [16]; phentolamine did not inhibit the effect of CPZ, but slightly potentiated it. It was indicated by Kunitada and Ui [16] that the release of endogenous catecholamines from the peripheral nerve terminals was responsible for the phentolamine-induced increase in plasma cyclic AMP, and enhancement by phentolamine of the plasma cyclic AMP rise induced by exogenous catecholamines was due to the blockade by phentolamine of post-synaptic α-adrenoceptors. Since CPZ behaves as an α-blocker in the CNS [3, 4], there is a possibility that CPZ increases plasma cyclic AMP by a mechanism similar to that of phentolamine at the adrenergic nerve terminals. However, our study, using adrenalectomy or 6-OHDA and the intracerebroventricular administration of CPZ, indicated that CPZ increases

plasma cyclic AMP by releasing catecholamines from the adrenal medulla.

The mechanism by which CPZ increases sympathetic activity by blocking the central α-adrenergic receptors is not known. Possible explanations are: (1) CPZ may occupy pre-synaptic α-adrenoceptors in the brain which inhibit sympathetic adrenergic activity, (2) CPZ may block norepinephrine binding to post-synaptic α-adrenoceptors in which norepinephrine may have an inhibitory character and, in turn, increase sympathetic tone directly or indirectly, or (3) stress by hypothermia induced by CPZ may stimulate sympathetic nerve activity.

It has been reported that the hyperglycemia induced by CPZ is more marked at lower rather than higher ambient temperatures, and that there is a correlation between the hyperglycemia and the hypothermia induced by CPZ [9]. There is a possibility, therefore, that CPZ increases plasma cyclic AMP by reducing body temperature. However our results clearly show that prevention of hypothermia by raising the room temperature did not significantly alter the cyclic AMP effect of CPZ, which excludes, therefore, the third possibility.

In conclusion, CPZ acts on the CNS and increases plasma cyclic AMP levels mainly by releasing catecholamines from the adrenal medulla.

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