EFFECT OF INTRACEREBROVENTRICULAR ADMINISTRATION OF CHLORPROMAZINE ON THE SERUM LEVEL OF FREE FATTY ACIDS IN RATS

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Abstract—The effect of intracerebroventricular administration of chlorpromazine (CPZ) on the serum level of free fatty acid (FFA) was studied in rats. Injection of CPZ into the lateral ventricle caused a transient decrease in serum FFA, showing the lowest level after 30 min. This decrease in serum FFA caused by CPZ was significantly inhibited by simultaneous injection of dopamine or apomorphine, although noradrenaline and serotonin had no effect. The dopaminergic blocking agent haloperidol caused a rapid decrease in serum FFA. After central chemical sympathectomy with the intracerebroventricular injection of 6-hydroxydopamine, the response to CPZ of serum FFA was completely abolished; but after peripheral sympathectomy by i.v. injection of 6-hydroxydopamine, a partial inhibition of the action of CPZ was shown. These results suggest a possible involvement of the central dopaminergic mechanism in the decrease in serum FFA after intracerebroventricular injection of CPZ.

There have been several studies concerning the effect of chlorpromazine (CPZ) on the lipolytic activity in vivo or in vitro, and between these studies, some discrepancy exists. In the in vivo studies, it has been shown that injection of CPZ results in an increase in plasma FFA in rats (1) and human subjects (2). However, Khan et al. (3) reported that CPZ prevented the elevation of plasma FFA levels in response to the stimuli of prolonged intermittent electric shocks, but did not alter basal plasma FFA levels in unshocked rats. Our previous result (4) was in good agreement with their report, i.e., CPZ has no influence on the serum level of FFA in normal rats. All these experiments were carried out after systemic administration of CPZ. In in vitro experiments, CPZ had no effect on the basal FFA release from epididymal adipose tissue (5) and FFA content in the tissue (1), but CPZ inhibited the catecholamine-induced lipolysis (5–7). Details of the influence of CPZ upon peripheral lipid mobilization are still obscure.

CPZ has also been thought to act by blocking dopamine receptors in the central nervous system (8–10), and the blockade of dopamine receptors by CPZ caused a compensatory marked acceleration of dopamine synthesis and release (11, 12). On the other hand, a great deal of evidence has been accumulated indicating that the ventromedial hypothalamus (VMH) may play an important regulatory role in the mobilization of FFA from peripheral adipose tissue (13–15).

Therefore, in an attempt to investigate a possible effect of CPZ on the central monoaminergic neurons involved in the regulation
of peripheral lipid mobilization, we have studied the changes in serum FFA after intracerebroventricular (i.c.v.) injection of CPZ in rats.

MATERIALS AND METHODS

Male rats of the Wistar-Imamichi strain, weighing 180–280 g, were used in these experiments. The environmental conditions were standardized (23±2 °C, humidity 55±5% and 14 hr artificial lighting per day) and water and pelleted food were supplied ad libitum.

For injections into the cerebral ventricles, a stainless steel cannula was implanted into the right lateral ventricle under sodium pentobarbital anesthesia (nembutal, 40 mg/kg i.p.) by the method of Noble et al. (16). After dissection of the head skin of the rats, an aperture with a 0.9 mm dia. was made in the skull with a dental drill. The coordinates were determined by measuring from 1.5 to 2.0 mm posteriorly and from 1.5 to 2.0 mm laterally to the right from the bregma. A short stainless steel cannula, 0.9 mm in outer dia., was introduced to a depth of 3.5 to 4.0 mm. The cannula was fixed to the skull with dental resin which also enveloped one small stainless steel screw inserted into the skull. To verify the position of the cannula tip, a 2% solution of pontamine sky blue was injected immediately before the animals were sacrificed, and the extent of the diffusion of the dye throughout the ventricles was confirmed. The implanted animals were maintained in individual cages.

After a 7-day post-operative recovery period, drugs were injected through this cannula without any anesthesia. All i.c.v. injections of drugs were made in the same volume (20 μl/kg), except for apomorphine and a high dose of haloperidol (200 μg/kg) which were in a double volume (40 μl/kg), over a period of 20 sec. Drug solutions were prepared as follows: chlorpromazine hydrochloride, dopamine hydrochloride, L-noradrenaline bitartrate, serotonin-creatine sulfate, and apomorphine hydrochloride were dissolved in saline and the solution was adjusted to a pH of 6.8 with 0.1 N sodium hydroxide. 2-Deoxy-D-glucose was dissolved in saline and some animals were given 500 mg/kg i.v. simultaneously with CPZ or saline. 6-Hydroxydopamine (6-OHDA) hydrobromide was dissolved in a saline solution containing 0.5% ascorbic acid immediately before injection. Two doses of 250 μg/rat of 6-OHDA were injected at an interval of 24 hr into the lateral ventricle 16 days before the experiments. Another group was given 10 mg/kg of 6-OHDA intravenously 4 hr prior to i.c.v. administration of CPZ or saline. All doses of drugs used are given in the results as the amount of free base.

Blood samples for determination of FFA were obtained from the jugular vein of rats with a disposable syringe. Serum FFA was determined colorimetrically according to the method of Novák (17).

Drugs were obtained from the following sources: Dopamine hydrochloride, L-noradrenaline bitartrate, apomorphine hydrochloride, 6-OHDA hydrobromide, and 2-deoxy-D-glucose from the Sigma Chemical Co.; serotonin-creatine sulfate from E. Merck; and haloperidol (Serenace) from the Dainippon Pharmaceutical Co., Ltd. All other reagents were of analytical grade.

RESULTS

After i.c.v. injection of CPZ, a significant transient decrease in serum FFA was produced with the two doses (200, 500 μg/kg) examined, whereas the same volume of vehicle (20 μl/kg) had no effect. With a lower dose of 100 μg/kg CPZ, only a slight decrease was observed; but this was not statistically significant. This temporary reduction in serum FFA reached its minimum at 30 min and was then restored to the initial level in about 2 hr.
(Fig. 1). Figure 2 shows the serum FFA and glycerol levels 30 min after i.c.v. injection of various doses of CPZ. The serum FFA at the minimum level was dose-related, but in contrast there were no significant changes in the serum glycerol level at any dose.

In order to investigate whether or not the CPZ-induced transient decrease in serum FFA was mediated by the central dopaminergic system, the effect of a simultaneous injection of dopamine (20 μg/kg i.c.v.) was studied (Fig. 3). The decrease in serum FFA after i.c.v. injection of 200 μg/kg CPZ was significantly inhibited by the simultaneous injection of dopamine (20 μg/kg) with CPZ into the right lateral ventricle, whereas dopamine alone did not show any significant change. These results suggest that dopamine

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**Fig. 1.** Effect of i.c.v. injection of CPZ on the serum FFA levels in rats. Various doses of CPZ (100, 200, 500 μg/kg) and vehicle were injected into the right lateral ventricle at 0 time. *: P<0.05, **: P<0.01 compared to the vehicle control. Throughout the figures in this paper, the following expressions hold: Figures in the parentheses represent the numbers of animals employed under each condition. The vertical lines indicate the S.E. of the mean. P values were calculated by the Student's t-test. *: P<0.05, **: P<0.01, ***: P<0.001.

**Fig. 2.** Effect of various i.c.v. doses of CPZ on the serum FFA and glycerol levels in rats at 30 min after i.c.v. injection.
counteracts the effect of CPZ.

Microinjection of either noradrenaline alone at a dose of 20 μg/kg or serotonin did not affect the serum concentration of FFA. The simultaneous administration of noradrenaline with CPZ into the right lateral ventricle did not produce any modification of the decrease in serum FFA after CPZ alone nor did serotonin produce any modification, suggesting that neither the noradrenergic nor the serotonergic neurons are involved in the effect of CPZ (Figs. 4, 5).

To test the effect of another dopaminergic agonist, apomorphine was injected intraventricularly. The i.c.v. injection of 100 μg/kg apomorphine alone did not produce any significant change in the serum FFA level, but the simultaneous injection of apomorphine

![Fig. 3](image1.png) **Fig. 3.** Effects of simultaneous injection of dopamine (20 μg/kg i.c.v.) on the decrease in serum FFA in response to CPZ (200 μg/kg i.c.v.). *: P<0.05 as compared to CPZ (200 μg/kg).

![Fig. 4](image2.png) **Fig. 4.** Effect of simultaneous injection of norepinephrine (20 μg/kg i.c.v.) on the decrease in serum FFA in response to CPZ (200 μg/kg i.c.v.).
(100 µg/kg i.c.v.) with CPZ (200 µg/kg i.c.v.) markedly suppressed the decrease in serum FFA induced by CPZ alone (Fig. 6).

One of the central dopaminergic blocking agents, haloperidol, also caused a rapid decrease in serum FFA. High doses (100, 200 µg/kg) of haloperidol were effective, whereas a dose of 20 µg/kg had no effect (Fig. 7).

Sixteen days after pretreatment with two i.c.v. doses of 250 µg 6-OHDA at an interval of 24 hr, the effect of CPZ (200 µg/kg i.c.v.) on the serum FFA was completely abolished. On the other hand, 4 hr after intravenous injection of 10 mg/kg 6-OHDA, the effect of CPZ (200 µg/kg i.c.v.) was only partially inhibited (Fig. 8).

In order to find out if i.c.v. CPZ influences...
the FFA mobilizing effect of 2-deoxy-D-glucose, which is known to be mediated by central-sympathoadrenal mechanisms (18–20), the effect of the i.c.v. injection of CPZ on the FFA mobilizing response to i.v. injection of 2-deoxy-D-glucose was further investigated. After the i.v. injection of 500 mg/kg 2-deoxy-D-glucose, a significant rapid increase in serum FFA was observed. The simultaneous injection of 200 μg/kg CPZ

Fig. 7. Effect of i.c.v. injection of haloperidol on the serum FFA level in rats. Various doses of haloperidol (20, 100, 200 μg/kg) and vehicle were injected into the right lateral ventricle at 0 time.

Fig. 8. Effect of pretreatment with 6-OHDA i.c.v. (central chemical sympathectomy) or i.v. (peripheral chemical sympathectomy) on the decrease in serum FFA in response to CPZ (200 μg/kg i.c.v.). Central chemical sympathectomy was performed by the i.c.v. injection of two doses of 250 μg/rat of 6-OHDA at an interval of 24 hr, 16 days before the experiments. Peripheral chemical sympathectomy was carried by i.v. injection of 10 mg/kg of 6-OHDA, 4 hr prior to i.c.v. injection of CPZ (200 μg/kg i.c.v.). *: P<0.05, **: P<0.01, ***: P<0.001 as compared to CPZ (200 μg/kg i.c.v.).
i.c.v. abolished this 2-deoxy-D-glucose-induced elevation of the serum FFA level (Fig. 9).

DISCUSSION

The injection of CPZ into the lateral ventricle caused a transient decrease in serum FFA in rats. This decrease in serum FFA was significantly inhibited by the simultaneous administration of dopamine or apomorphine, whereas noradrenaline and serotonin had no effect on the level of serum FFA. It is therefore demonstrated from these data that the effect of CPZ on the peripheral concentration of FFA is closely related to the central dopaminergic system.

After central chemical sympathectomy with i.c.v. injection of 6-OHDA, the response to CPZ in serum FFA was completely abolished; but after peripheral chemical sympathectomy, only a partial inhibition of CPZ action was observed. As it is well known that 6-OHDA does not penetrate through the blood brain barrier in the adult animal, its intravenous injection may not cause any change in the central catecholamine level. However, Iversen and Uretsky (21) reported that after the central treatment with two doses of 250 μg 6-OHDA, dopamine concentrations in the striatum and noradrenaline concentrations in the hypothalamus were reduced to 12.1% and 14.8% of the control values, respectively. Therefore, our results indicate the possibility that the effect of i.c.v. CPZ on the level of serum FFA might be related to central dopaminergic neurons or noradrenergic neurons.

Several reports indicated that the inhibition of glucose metabolism by the systemic administration of 2-deoxy-D-glucose, which is known as a competitive inhibitor of glucose uptake, results in an activation of the sympathetic axis leading to an increase in the level of circulating FFA (18–20). Some other reports using hypothalamic deafferented rats postulate an involvement of the anterior hypothalamus or the limbic system in controlling the peripheral FFA mobilization (22, 23). Our results confirmed the effect of 2-deoxy-D-glucose to increase the serum FFA concentration and demonstrated that this effect of 2-deoxy-D-glucose was blocked by i.c.v. injected CPZ. This inhibitory effect of i.c.v. CPZ also indicated the central effect
of CPZ on fat mobilization.

Recently studies on the binding properties of the dopamine receptor, using (3H)-haloperidol as a dopamine antagonist, indicated that CPZ binds specifically to postsynaptic dopamine receptors (24-27) and that this blockade presumably leads to decreased activity of the neuronal dopamine pathways (9, 28, 29). Furthermore, the report that CPZ inhibited the electrically stimulated release of (3H)-dopamine from rat striatal slices shows that this drug also has a presynaptic action on dopamine neurons (30).

On the other hand, a good deal of evidence has accumulated to show that the ventromedial hypothalamus (VMH) may play a very important integrative and regulatory role in peripheral lipid mobilization. Kumon et al. (13) reported that electrical stimulation of the VMH in rabbits increased the plasma glycerol concentration, indicating acceleration of lipolysis in adipose tissues. Furthermore, Bray and Nishizawa (14, 15) showed that both electrolytic lesions of the VMH and sympathetic denervation in rats impaired the mobilization of fatty acids from the adipose tissue caused by fasting or by subjecting the animals to stress such as cold exposure, swimming, or injection of norepinephrine and 2-deoxy-D-glucose. The hypothalamic deafferentation (22, 23) also impaired FFA mobilization induced by sympathetic activation. These results indicated that the VMH is involved in the regulation of FFA release caused by the sympathetic activation. The VMH may act as a modulator of peripheral efferent sympathetic activity, particularly that involved in regulating the adipose tissue mass.

There is also histological evidence that the VMH is a part of the anatomic organization of the sympathetic neural output from the hypothalamus (31, 32). Thus it might be considered that the VMH is one of the most probable sites of the influence of CPZ on FFA mobilization. However, according to a minireview about the neuroendocrine control of appetite by Morley (33), the VMH which known as a satiety center is not apparently associated with the dopaminergic tract, although the adjacent lateral hypothalamic feeding center appears to be closely connected with the nigrostriatal dopaminergic bundle, i.e., some nerve fibers originating from the substantia nigra terminate at the lateral hypothalamus (LH) and some others pass through this area and extend the projections to the basal ganglia. The anatomically close connection between the VMH and the LH has been indicated (34-36), and intensive electrophysiological studies demonstrated the characteristics of the chemosensitive neurons of the VMH and the LH (37), substantiating their well established reciprocal function in the regulation of food intake (33, 38) and peripheral metabolism (39). Thus, it may be postulated that CPZ first affects the dopaminergic neurons involving the nigrostriatal system which in turn affects LH through an interaction with VMH. To clarify details of the mechanism of the CPZ action, further studies are required.

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