HYPERGLYCEMIC ACTION OF 2-(2,6-DICHLORPHENYL-AMINO)-2-IMIDAZOLINE HYDROCHLORIDE IN RELATION TO ITS HYPERTENSIVE EFFECT

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Hoefke and Kobinger reported that 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, ST-155 (Fig. 1), had a long-lasting hypotensive action in dogs and rabbits (1). Then Kobinger and Walland (2), and Toda *et al.* (3) also observed the similar results in animal experiments. The mechanism of its action, however, has not been thoroughly understood. Hoefke and Kobinger suggested that the hypotensive action of ST-155 might be due to the inhibition of the center of sympathetic nervous system as well as to the modi-

FIG. 1. Chemical structure of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (ST-155).

fication of baro- and chemoreceptor reflex (1). From the fact that continuous intracerebroventricular infusion of minute doses of ST-155 (0.1—1.0 $\mu\text{g/kg}$) lowered the systemic blood pressure in rabbits, Toda *et al.* supposed that its hypotensive action could be mediated mainly by the central nervous system. But, they also mentioned that the hypotensive action of ST-155 was not explained solely by its action on the central nervous system, since the pretreatment with hexamethonium or guanethidine could not abolish this action (3).

It is remarkable that this drug shows a significant difference by species. In rabbits, small doses of ST-155 lower the blood pressure, but the hypotensive action of this drug is not manifestly observed in cats and rats. On the contrary, the efficient anti-hypertensive doses in rabbits raise the blood pressure in cats and rats.

Moreover, it is reported that this drug has a hyperglycemic action and that the species difference is also observed in this hyperglycemic action as in the hypotensive effect (1, 3).

In the present report, the mechanism of the hyperglycemic action of ST-155 has been analyzed in rats, since a hyperglycemic response of rats to ST-155 is much more significant than other animals, and the relationship between the effect of this drug on blood pressure and blood sugar has been discussed.

MATERIALS AND METHODS

1. Examination of blood sugar

Rats: In order to obtain a dose-response curve and study the mechanism of the action of ST-155, Wistar strain male rats weighing 60 to 80 g were used. Rats were fed for the first week on a standard diet (MF, Oriental Co.) and for the next 10 days before experiments on the standard food containing high protein and fat by the Sato and Takeuchi's method (4). Water was given *ad libitum* throughout the preexperimental period and during experiments. Animals were fasted for 16 hours prior to experiments and maintained at $32\pm 2^\circ\text{C}$ room temperature during the fasting and experimental period. Blood sample (0.02 ml) was taken from the tail vein just before and at 1, 2, 3, 5 and 7 hours after the administration of the test drug, ST-155, for the determination of blood sugar level.

Bilateral adrenalectomized rats were prepared according to the method described by Ingle and Griffith (5). They were kept at $32\pm 2^\circ\text{C}$ and given 0.9% sodium chloride solution instead of water after operations and during the experiment. They also were subcutaneously administered 1 mg/animal/day of DOCA for 2 days and used on the third day after the operation.

Adrenaline hydrochloride was used in order to compare with the action of ST-155, and the drugs used for the study of the site of its action were hexamethonium bromide, reserpine (Serpasil), propranolol hydrochloride and dihydroergotamine methanesulfonate. Each drug was dissolved in a saline solution and was subcutaneously injected on the back except propranolol, which was given into the tail vein. All solutions of drugs were prepared just before each experiment. The volume of a single injection was 0.2 ml intravenously and 0.5 ml subcutaneously.

Rabbits: Rabbits weighing 2.2 to 2.7 kg were used for obtaining a dose-response curve, after feeding them for two weeks on a standard diet (RC5, Oriental Co.) and fasting them 16 hours prior to experiments. Water was given *ad libitum* throughout the preexperimental and experimental period. Blood sample (0.1 ml) was taken from the ear vein for a determination of blood sugar level. The test drug, ST-155, was dissolved in a saline solution and injected subcutaneously on the back, and the volume of a single injection was 5 ml.

In the present study, each experimental group consisted of 4 to 8 animals, and the blood sugar level was determined by glucose-oxidase method. Statistical analysis of the results from the present experiments, that is, significant test on group comparison and linearity test on regression curve of response to log-dose, were based on the variance analysis method introduced by Finney (6).

2. Examination of blood pressure

Wistar strain male rats weighing 200 to 250 g and rabbits weighing 2.5 to 3.0 kg were used. Each experimental group consisted of 3 to 4 animals.

Arterial blood pressure was measured from the right carotid artery, by using a pressure-transducer and a pen-writing oscillographic recorder under urethane anesthesia (1.5

g/kg s.c.). Simultaneously, respiratory movements and electrocardiogram (Lead II) also were recorded.

Each drug was dissolved in a saline solution and injected through a polyethylenic canula, inserted into left femoral vein in rabbits and left carotid vein in rats, in about 10 seconds.

RESULTS

1. Effect on blood sugar

Rats: When ST-155 (10–100 $\mu\text{g/kg}$) was administered subcutaneously in rats, at doses of 10 $\mu\text{g/kg}$, a significant rise in blood sugar was observed, reaching the maximum 2 hours later and returning to the initial levels in about 5 hours. This hyperglycemic response was proportionally intensified as the dose of the drug was increased within the range of doses above mentioned (Fig. 2).

FIG. 2. Hyperglycemic response induced by ST-155 in rats. Control group was subcutaneously injected 0.5 ml of a saline solution. Each point represents the mean value for 8 animals, and vertical lines indicate the standard deviations.

FIG. 3. Regression curve of hyperglycemic response to ST-155 in rats. The index of hyperglycemic response is expressed as sums of blood sugar values determined during 5 hours. Shaded area indicates ± 1 S.D. of the mean value in control group.

Fig. 3 shows a dose-response curve of hyperglycemic response induced by ST-155 in rats. There is a linear regression (regression coefficient $b: 157.3$, linear regression: $F=40.2 > F_{21}=8.02$ ($P < 0.01$)) in a range of doses above described.

When 20 mg/kg of hexamethonium was administered 15 minutes before and 1, 2 and 3 hours after the administration of 30 $\mu\text{g/kg}$ of ST-155, a significant change in hyperglycemic response was not found (Fig. 4a). And the group, which was pretreated with 1 mg/kg of reserpine 16 hours prior to ST-155 administration, also showed no appreciable change in this hyperglycemic response (Fig. 4b).

Fig. 4 a. Effect of the pretreatment with hexamethonium (20 mg/kg s.c.) on hyperglycemia induced by ST-155 (30 μ g/kg s.c.) in rats. Hexamethonium was administered 15 minutes before and 1, 2 and 3 hours after ST-155 administration. Each point represents the mean value for 6 animals (left).

b. Effect of the pretreatment with reserpine (1 mg/kg s.c.) on hyperglycemia induced by ST-155 (30 μ g/kg s.c.) in rats. Reserpine was administered 16 hours prior to ST-155 administration. Each point represents the mean value for 6 animals (right).

On the other hand, the pretreatment with 3.5 mg/kg of dihydroergotamine administered 15 minutes prior to the injection of 30 μ g/kg of ST-155 could completely abolish the hyperglycemic response induced by ST-155. When 30 μ g/kg of ST-155 was admin-

Fig. 5 a. Effect of the pretreatment with propranolol (0.25 mg/kg i.v.) on hyperglycemia induced by ST-155 (30 μ g/kg s.c.) in rats. Propranolol was administered 45 minutes prior to ST-155 administration. Each point represents the mean value for 6 animals (left).

b. Effect of the pretreatment with dihydroergotamine (3.5 mg/kg s.c.) on hyperglycemia induced by ST-155 (30 μ g/kg s.c.) in rats. Dihydroergotamine was administered 15 minutes prior to ST-155 administration. Each point represents the mean value for 6 animals (right).

Fig. 6 a. Effect of the pretreatment with dihydroergotamine (3.5 mg/kg s.c.) on adrenaline hyperglycemia in rats. Each point represents the mean value for 6 animals (left).
b. Effect of the pretreatment with reserpine (1 mg/kg s.c.) on adrenaline hyperglycemia in rats. Each point represents the mean value for 6 animals (right).

istered 45 minutes after the intravenous administration of 2.5 mg/kg of propranolol its hyperglycemic response was significantly inhibited (34.6%, $P < 0.05$), but not so completely as when pretreated with dihydroergotamine (Fig. 5a and 5b).

The pretreatment with 1 mg/kg of reserpine caused no definite effect on hyperglycemic response induced by 0.1 mg/kg of adrenaline, but the pretreatment with 3.5 mg/kg of dihydroergotamine did completely abolish it (Fig. 6a and 6b).

Fig. 7 shows per cent increase of the blood sugar levels after administration of ST-155 in normal and adrenalectomized rats. A significant rise in blood sugar ($P < 0.05$) was observed even in bilateral adrenalectomized rats, while the hyperglycemic response was by about 44% less than that in non-treated group.

Rabbits: In rabbits, the administration of 10 to 100 $\mu\text{g/kg}$ of ST-155 produced no significant rise in blood sugar. Remarkable hyperglycemic responses were observed in doses of more than 300 $\mu\text{g/kg}$, but at the same time, there were marked differences among the experimental animals. Consequently, a linear regression between doses of ST-155 and the responses of animals was non-significant.

Fig. 7. Per cent increase of blood sugar levels in normal and adrenalectomized rats following ST-155 (30 $\mu\text{g/kg}$ s.c.) administration. Each point represents the mean value for 5 animals.

2. Effect on blood pressure

Rats: When 10 $\mu\text{g/kg}$ and 30 $\mu\text{g/kg}$ of ST-155 were intravenously injected into rats, a transient rise in blood pressure was produced immediately after injection. At the same

FIG. 8. Effect of intravenous administration of ST-155 on blood pressure in rat before and after the pretreatment with dibenamine.

TABLE 1. Effect of intravenous administration of ST-155 on blood pressure in rats before and after the pretreatment with dibenamine (15 mg/kg). Dibenamine was administered 45 minutes prior to ST-155 administration.

Dose	No. of animals	Maximum change in blood pressure (%) (Mean $\pm$ S.E.*)	
		Before treatment	After treatment
ST-155 10 $\mu\text{g/kg}$	3	+50.3 $\pm$ 15.3	+12.9 $\pm$ 0.37
ST-155 30 $\mu\text{g/kg}$	3	+76.5 $\pm$ 19.9	+32.7 $\pm$ 7.3

* $\pm$ S.E. : standard error of the mean

TABLE 2. Effect of intravenous administration of ST-155 on blood pressure in rabbits.

Dose	No. of animals	Maximum change in blood pressure (%) (Mean $\pm$ S.E.*)
ST-155 10 $\mu\text{g/kg}$	4	-24.9 $\pm$ 1.8
ST-155 30 $\mu\text{g/kg}$	4	-36.4 $\pm$ 2.8
ST-155 100 $\mu\text{g/kg}$	2	-50.4
ST-155 1 mg/kg	4	+33.1 $\pm$ 7.2

* $\pm$ S.E. : standard error of the mean

time, frequency of respiration decreased, but the change in heart rate was not obviously observed. The pretreatment with 15 mg/kg of dibenamine administered 45 minutes prior to the administration of ST-155 suppressed the rise in blood pressure induced by ST-

FIG. 9. Effect of intravenous administration of ST-155 on blood pressure in rabbit.

FIG. 10. Effect of the pretreatment with dibenamine on a rise in blood pressure induced by ST-155 (1 mg/kg i.v.) in rabbit.

155, but had no influence on the decrease in frequency of respiration (Fig. 8 and Table 1).

Rabbits: Intravenous administration of 10 to 100 $\mu\text{g/kg}$ of ST-155 always produced a long-lasting decrease in blood pressure in rabbits. Standard deviation of a fall of blood pressure in individual animal was found relatively large, but regarding the mean values, it was evident that there was a definite correlation between doses of ST-155 and the hypotensive responses of animals. The administration of 10 $\mu\text{g/kg}$ and 30 $\mu\text{g/kg}$ of ST-155 produced little effects on heart rate and frequency of respiration. Following the administration of 100 $\mu\text{g/kg}$ of ST-155, however, the diminution of heart rate was observed, and frequency of respiration showed a tendency to increase, being accompanied with the reduction of amplitude (Fig. 9 and Table 2).

On the other hand, intravenous administration of 1 mg/kg of ST-155 caused a transient rise in blood pressure, reaching the maximum immediately after injection, and a marked decrease in heart rate also was observed. Frequency of respiration was either increased or decreased, but reduction of amplitude of respiration was consistent; apnea was observed in 3 out of 4 animals. When pretreated with 15 mg/kg of dibenamine, a transient rise in blood pressure occurring immediately after injection was markedly suppressed, and blood pressure tended gradually to increase. And the reduction of amplitude of respiration was definitely decreased (Fig. 10).

DISCUSSION

It is widely accepted that adrenaline has a direct action on liver and produces a rise in blood sugar, that is, adrenaline hyperglycemia, by increasing glucose output from liver. According to Sutherland and Ball, adrenaline activates a formation of cyclic 3', 5'-AMP in liver and this nucleotide converts inactive phosphorylase b to active phosphorylase a, which stimulates glycogenolysis and increases glucose output from liver (7).

It has long been known that morphine has a hyperglycemic action. Borison *et al.* supposed that morphine produced hyperglycemia reflexly by its action on a paraventricular receptor site probably located near the Foramen of Monro (8). It has generally been accepted that morphine produces hyperglycemia by increasing adrenaline discharge from suprarenal gland via the central nervous system.

The hyperglycemic action of ST-155 is probably due to a increase in glucose output from liver either by its central or peripheral action; four possible mechanisms are involved: 1) stimulation of central nervous system; 2) stimulation of the periphery of sympathetic nerve; 3) promotion of a discharge of adrenaline from medulla of suprarenal gland; and 4) increase of a glycogenolysis in liver.

Hexamethonium is considered to have the action of inhibiting adrenaline discharge, as well as the blocking action of autonomic ganglia, affecting the synapse of sympathetic nerves distributed to suprarenal gland. And the effects of some drugs, which are supposed to have an action to stimulate the release of endogenous noradrenaline, were reduced in animals pretreated with reserpine, since this compound depletes noradrenaline stores at the endings of postganglionic fibers in sympathetic system (9).

The fact that the pretreatment with hexamethonium or reserpine has no influence on the hyperglycemic response to ST-155, may suggest that neither the central nervous system nor the sympathetic nervous system participates in this hyperglycemic response.

It has already been reported that the administration of ST-155 caused a significant rise in blood sugar even in bilateral adrenalectomized rats, although the hyperglycemic response in adrenalectomized animals was smaller than that in control animals (1, 3). These observations seem to coincide with the present results. Toda *et al.* supposed that hyperglycemic response to ST-155 was produced not only by extrasuprarenal factors, but also by suprarenal mechanism. But, in the present experiment, it seems that the insufficiency in adrenocorticosteroids, especially glucocorticoides, induced by bilateral adrenalectomy may play an important role in diminishing the hyperglycemic responses induced by ST-155. When food is continuously given, no remarkable abnormality in carbohydrate metabolism is observed even after adrenalectomy; normal blood sugar levels can be maintained and glycogen is stored in liver. However, it is well known that the rapid decrease in the concentration of glycogen in liver and the fall in blood sugar are observed under starvation of a brief period. In such cases, administration of glucocorticoid such as cortisol restores carbohydrate metabolism; normal blood sugar levels are maintained even during fasting. Also, the increase of nitrogen in urine indicates that protein is converted to carbohydrate. It is recently reported that cortisol itself may activate glucogenesis by its direct effect on carbohydrate metabolism (10). Considering these facts, the present results seem to tell that the decrease of hyperglycemic response to ST-155 in adrenalectomized rats can be attributed to the diminution of storage of glycogen in liver caused by the insufficiency of adrenocorticosteroids.

It has long been known that dihydrated ergot alkaloids inhibit adrenaline hyperglycemia more effectively than the other α -adrenergic blocking agents. These alkaloids are supposed to antagonize the formation of cyclic 3', 5'-AMP induced by adrenaline in liver cells *in vitro*, but not that induced by glucagon (7). Tobe *et al.* reported that the hyperglycemic response in rats induced by the administration of 0.2 mg/kg of adrenaline was completely blocked by the pretreatment with 3.5 mg/kg of dihydroergotamine (11).

There are many reports that the effects of catecholamines on carbohydrate metabolism can be antagonized by β -adrenergic blocking agents. According to Kvam *et al.*, β -adrenergic blocking agents such as pronethalol, MJ 1999 and MJ 1998 inhibited adrenaline hyperglycemia in dogs (12). It is also reported that propranolol, one of β -adrenergic blocking agents, antagonized adrenaline hyperglycemia in dogs (13).

The present results, as above mentioned, might show the possibility that this hyperglycemic action of ST-155 results from its direct action on liver itself, and is not mediated by the central nervous system. Moreover, it may be suggested that the hyperglycemic action of ST-155 resembles that of adrenaline and probably results from its sympathomimetic action, since this action is antagonized by the pretreatment with propranolol or dihydroergotamine.

Hoefke and Kobinger reported that intravenous administration of ST-155 (10—100

$\mu\text{g/kg}$) always produced a long-lasting fall in blood pressure preceded by a slight transient rise in blood pressure in rabbits. Toda *et al.* also obtained the similar results in doses of 5 to 50 $\mu\text{g/kg}$. In the present experiment, in doses of 10 to 100 $\mu\text{g/kg}$ of ST-155, a long-lasting fall in blood pressure was always observed in rabbits, though the preceding transient rise in blood pressure was not manifest. The administration of 1 mg/kg of ST-155, however, caused no fall in blood pressure, only producing a transient rise in blood pressure. On the other hand, in rats, in doses of 10 $\mu\text{g/kg}$, only a transient rise in blood pressure was seen without a fall in blood pressure.

It is now generally supposed that the hypotensive action of ST-155 was mediated by the central nervous system. Naylor *et al.* suggested, in the experiment using the dog heart-lung bypass preparations (14), that adrenergic β -receptors took part in the hypotensive action of ST-155 (15). But, Toda *et al.* concluded that adrenergic β -receptors played no role in its hypotensive action at least in rabbits, because its action was not influenced by the pretreatment with propranolol in anesthetized rabbits.

It was reported that the transient hypertensive effect in dogs and cats, induced by intravenous administration of ST-155, was diminished or abolished when pretreated with α -adrenergic blocking agent, but not when pretreated with reserpine (2, 3). These facts may show that the hypertensive action of ST-155 in dogs and cats can be a direct effect on adrenergic α -receptors. In the present experiment, the pretreatment with dibenamine, an α -adrenergic blocking agent, also significantly inhibited the rise in blood pressure in rats, and diminished the transient rise in blood pressure occurring immediately after the injection of 1 mg/kg of ST-155 in rabbits. These results indicate the possibility that the rise in blood pressure observed in rats and rabbits also is due to a direct action of ST-155 on adrenergic α -receptors, as is the case with dogs and cats.

Comparing the effect of ST-155 on blood pressure with that on blood sugar, both in rats and rabbits, the doses that can produce a rise in blood sugar also can produce a rise in blood pressure, but the efficient doses that can lower the blood pressure do not raise the blood sugar. This fact may also suggest that the hypertensive action of ST-155 can be ascribed to its sympathomimetic action as in hyperglycemic action.

The fact that the administration of 10 to 100 $\mu\text{g/kg}$ of ST-155 lowers blood pressure in rabbits, but increases it in rats, might show that a direct effect of ST-155 on adrenergic α -receptors overcomes its hypotensive action in the latter. So the species differences observed in effects of ST-155 on blood pressure and blood sugar may depend on the susceptibility of animals to its sympathomimetic action.

SUMMARY

In order to explain the mechanism of hypotensive action of ST-155, the mechanism of its hyperglycemic action has been analyzed and the relationship between the effect on blood pressure and blood sugar has been discussed.

The hyperglycemic action of ST-155 in rats was not influenced by the pretreatment with hexamethonium or reserpine, but significantly inhibited when pretreated with pro-

pranolol or dihydroergotamine. So the hyperglycemic action might be resulted from the sympathomimetic action of ST-155.

In rats and rabbits, the administration of the doses, which can increase the blood sugar, caused the rise in blood pressure. This may show that the hypertensive effect of that doses observed in rats and rabbits comes from its sympathomimetic action, especially from its direct action on adrenergic α -receptors.

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