Mechanism of Hypotensive Action of 2-(2,6-Dichlorophenylamino)-2-imidazoline Hydrochloride (ST-155) in the Cat

By R. D. MAGUS* and J. P. LONG

A study of the mechanism of hypotensive and bradycardic action of ST-155 in the cat revealed that this action is in part related to a centrally mediated bradycardia. Bradycardia and the hypotensive action of ST-155 were antagonized or abolished by hexamethonium, bretylium, decerebration, or by stellate ganglionectomy. The action of ST-155 does not appear to involve veratrine-sensitive pathways inasmuch as denervation of the carotid sinus or excision of the nodose and superior cervical ganglia were without effect on the action of ST-155. ST-155 in the dosage used (8 mcg./Kg.) potentiated the pressor responses to epinephrine and tyramine. It is presumed that reduced central sympathetic transmission to the heart from the cardiac sympathetic nerves is a major mechanism for the hypotension and bradycardia following ST-155 administration.

THE TOLAZOLINE ANALOG 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (ST-155) (Fig. 1) is currently being widely investigated in both animals and man as a possible antihypertensive agent. Studies in animals to date indicate that ST-155 administered intravenously exhibits a dose-dependent (1–100 mg./Kg.) reduction in heart rate and blood pressure in vagotomized dogs, an effect which is preceded by a brief pressor response (1). In addition,

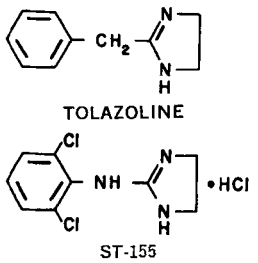

Fig. 1—Structures of ST-155 and tolazoline.

ST-155 has been shown to depress the carotid sinus reflex, reduce cardiac output with little change in peripheral resistance during the hypotensive phase, and exhibit a variety of sympathomimetic actions (*e.g.*, vasoconstriction in the isolated rabbit ear, splenic contraction in cats, increase in contraction amplitude of isolated spontaneously beating guinea pig atrial) (1). With relatively larger doses in rats ST-155 has been shown to increase urine volume and electrolyte excretion although such effects have not been seen in dogs receiving the drug in doses which produced marked hypotensive actions (1).

Clinical studies have thus far shown that ST-155 in microgram dosage is an orally effective antihypertensive agent in man with a low incidence of side effects (2–7) and toxicity (*loc. cit.*, 8). For example, orthostatic hypotension is a minor occurrence with therapeutically effective doses; however, fatigue, sedation, and anticholinergic effects have been repeatedly observed in humans receiving ST-155 (2–7).

To date, the mechanism of hypotensive action of ST-155 has not been elucidated (9). The present experiments were, therefore, undertaken in an attempt to provide a possible mechanism of hypotensive action of ST-155 in the anesthetized cat. The studies appear to indicate that a major component of the drug's hypotensive action is a centrally mediated inhibition of sympathetic cardioaccelerator nerves resulting in bradycardia.

EXPERIMENTAL

Animals—Thirty-five adult female mongrel cats weighing between 1.4 and 3.2 Kg. were used in this study. Anesthesia was performed by intrathoracic administration of sodium pentobarbital (30–35 mg./Kg.). All animals were tracheotomized and artificially respired on a Palmer respirator. Blood pressures were monitored, *via* a heparinized cannula inserted in the femoral artery, by a Statham arterial pressure transducer and recorded on an Offner RS dynograph. Heart rate was monitored with a stopwatch for 15-sec. periods at the intervals indicated on the charts. Injections of test drugs or infusion of additional anesthetic, when required, were made into the contralateral femoral vein either directly or through a cannula. Other operative procedures are described in the text where appropriate.

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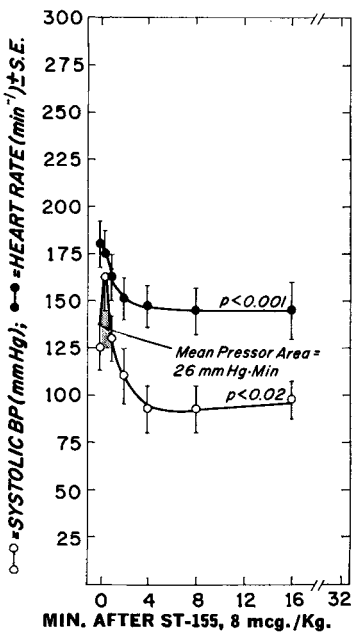

Fig. 2—Blood pressure and heart rate following ST-155 (8 mcg./Kg.) in the anesthetized cat. Key (Figs. 2–8): O, systolic blood pressure; ●, heart rates; S.E. = standard error of the mean; pressor areas are defined as the areas (mm. Hg × time) under the pressor phase of the systolic pressure curve. Statistical comparisons are made between the 0 time and 16-min. point.

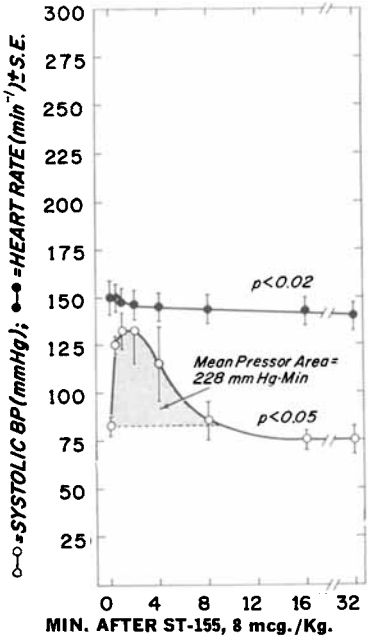

Fig. 3—Effect of hexamethonium pretreatment (10 mg./Kg.) on ST-155 action.

Drugs—ST-155 was administered as the water-soluble hydrochloride prepared in ampul form.[1] Tyramine hydrochloride (Eastman), hexamethonium chloride (Nutritional Biochemicals), and bretylium tosylate (Burroughs Wellcome) were administered in 0.9% saline solution. Epinephrine and isoproterenol were diluted before use from concentrated solutions (Parke-Davis). All dosages are expressed as the salt. Bretylium was administered by slow intravenous infusion; hexamethonium was similarly infused after an initial fraction of the administered dose was given intramuscularly. All other drugs were given by rapid intravenous injection.

In each experiment a total of five cats was used. All data are expressed as mean ± standard error; Student's 2-tailed *t*-test (paired comparison) was employed as described by Steel and Torrie (10).

RESULTS

Hypotensive Action of ST-155 in the Cat—The time course of pressor and depressor responses and bradycardia following 8 mcg./Kg. ST-155 administered intravenously is presented in Fig. 2. This dosage of ST-155 was selected from preliminary experiments as a dose which reduced heart rate and systolic pressure by approximately 20–25% and was used routinely in all subsequent experiments. The response to ST-155 in the cat (vagi intact) shown in Fig. 2 illustrates the initial transient pressor phase (30% increase in systolic blood pressure) concomitant with bradycardia. It will be noted that both the heart rate and arterial blood pressure 16 min. after administration of ST-155 are significantly different from preinjection values.

Effect of Hexamethonium Pretreatment on ST-155 Activity—In order to test the possibility that hypotension and bradycardia from ST-155 were reflexogenic, cats (vagi intact) pretreated with hexamethonium (10 mg./Kg.) were given ST-155 (8 mcg./Kg.) after the base line blood pressure following hexamethonium had become reasonably stable (20–30 min.).

The data in Fig. 3 indicate that hexamethonium pretreatment markedly reduced the bradycardia and hypotensive action of ST-155 and greatly potentiated its initial pressor action. Indeed, there is a ninefold increase in the pressor area (duration × pressure) when a comparison is made with cats not receiving hexamethonium pretreatment (Fig. 3 *versus* Fig. 2). A slight, albeit statistically significant change from pre-ST-155 values in heart rate and systolic blood pressure is seen at 16 min. However, since these changes vary only 7–8% from pre-ST-155 values and may be due to the action of hexamethonium, it may be concluded that bradycardia and hypotension due to the action of ST-155 are associated with a reflex. It would thus seem possible that hexamethonium abolished a depressor reflex which is otherwise triggered by ST-155.

Cardiovascular Response to ST-155 in the Decapitated Cat—That the hypotensive response to ST-155 was centrally mediated was tested by administration of the drug to cats that had a large press clamp placed tightly around the neck centrally to the tracheal cannula. This procedure effectively severed any functional connection of the head to the remainder of the animal. It was seen that such a maneuver abolished any depressor action of ST-155 (8 mcg./Kg.) although a transient bradycardia was seen (Fig. 4). However, no significant difference in both parameters was seen 16 min. after ST-155 administration. Whereas the pressor response to ST-155 showed a 30% increase in systolic pressure as in the intact cat (Fig. 4 *versus* Fig. 2), this action

[1] Catapres, Geigy.

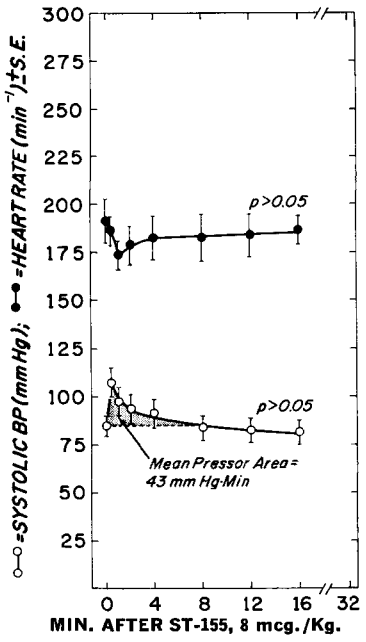

Fig. 4—Response to ST-155 in the headlamped cat.

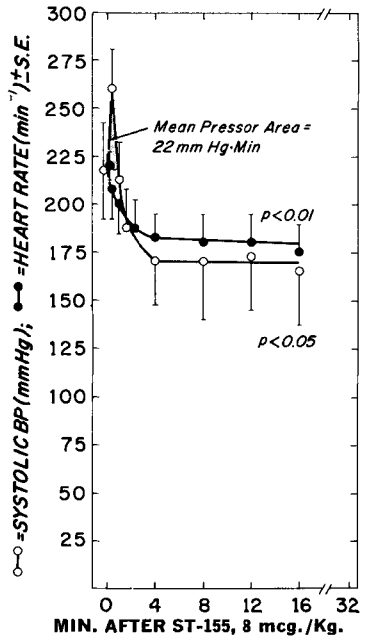

Fig. 5—Response to ST-155 following carotid sinus denervation.

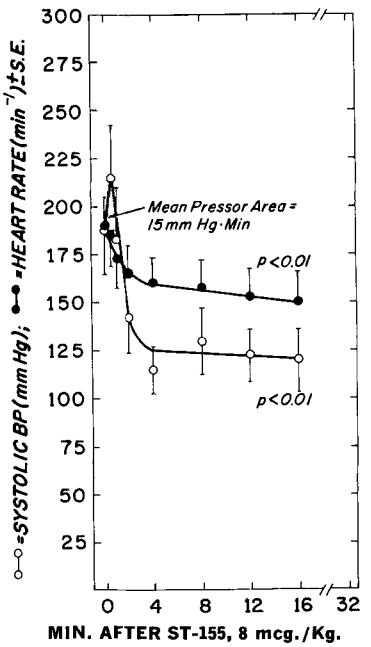

Fig. 6—Response to ST-155 following carotid sinus denervation and superior cervical and nodose ganglionectomy.

was of slightly longer duration in the headless cat (43 mm. Hg min. *versus* 29 mm. Hg min. pressor area). It would therefore appear that bradycardia and hypotension following ST-155 occur through centrally mediated pathways.

Effect of Carotid Sinus Denervation Alone or with Nodose and Superior Cervical Ganglionectomy on ST-155 Action—The possibility that ST-155 was activating reflex hypotension in a manner analogous to that of veratrum alkaloids (11, 12) was examined in the following experiments. Carotid sinuses were denervated bilaterally and painted with 1% lidocaine solution. Such a maneuver did not alter the action of ST-155 (Fig. 5 *versus* Fig. 2), nor did excision of the nodose ganglia and the superior cervical ganglia in addition to carotid sinus denervation (Fig. 6 *versus* Fig. 2). It was apparent, therefore, that the locus of action of ST-155 was unlike veratrum-like agents which appear to act reflexly *via* an action on the baroreceptor buffering mechanism and nodose ganglia (12).

Response to ST-155 After Bretylium Pretreatment or Stellate Ganglionectomy in Vagotomized Cats—It was speculated that a possible mode of action of ST-155 was *via* reflex inhibition of the cardiac sympathetic fibers emanating from the stellate ganglion. Pretreatment of bilaterally vagotomized cats with bretylium (15 mg./Kg.) (on the premise that if sympathetic nerve transmission was already inhibited by this means then ST-155 would not show hypotensive activity), potentiated the pressor action of ST-155 (8 mcg./Kg.), abolished the bradycardia, and markedly reduced the depressor action to a 10% change (at 16 min.) from pre-ST-155 values (Fig. 7). This effect of bretylium was much like that seen earlier with hexamethonium pretreatment (Fig. 3). Furthermore, administration of ST-155 (8 mcg./Kg.) to bilaterally vagotomized cats, in which the stellate ganglia were removed following a midline incision along the sternum, produced only a pressor response (Fig. 8). Again, the pattern of the response resembled that seen in the animals pretreated with hexamethonium (*cf.* Fig. 3).

Effect of ST-155 on Cardiovascular Reactivity to Tyramine, Epinephrine, and Isoproterenol—An investigation of the action of ST-155 was performed in order to examine its action at the adrenergic receptor level. Previous reports (1) indicated that with extremely high doses ST-155 may have adrenergic blocking activity. This would not be unexpected in view of its structural resemblance to tolazoline (Fig. 1). However, it was found that ST-155 in an 8 mcg./Kg. dose appeared to potentiate the pressor actions of epinephrine in both the intact and headless cat and of tyramine in the intact cat; the vasodepressor action of isoproterenol in the intact cat was not affected by ST-155 pretreatment

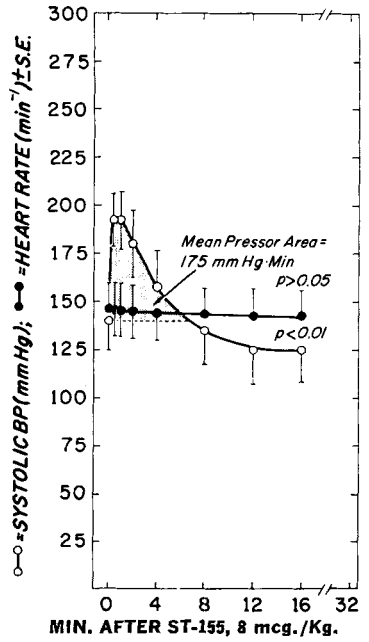

Fig. 7—Effect of bretylium pretreatment (15 mg./Kg.) on ST-155 action.

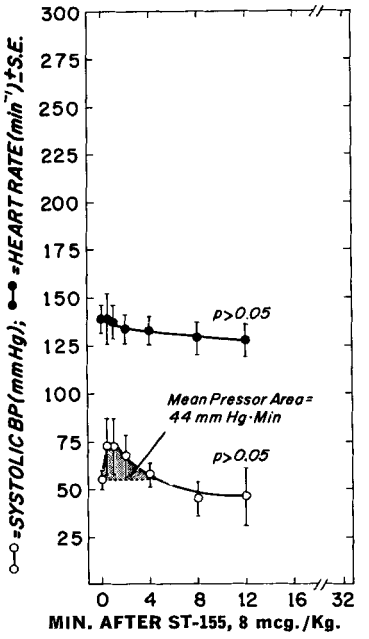

Fig. 8—Response to ST-155 following bilateral vagotomy and stellate ganglionectomy. No measurements were made beyond the 12-min. point.

(Fig. 9). It is apparent, therefore, that adrenergic blockade by ST-155 was not operative in the dosage used in this study.

DISCUSSION

If ST-155 were acting by central inhibition of the cardiac sympathetic fibers, the results obtained in this study would be in agreement with such a mechanism. Inasmuch as only a partial blockade of hypotension was obtained with bretylium and hexamethonium, it is difficult to state unequivocally that this is the mechanism of action. Such conclusions are further complicated by the possibility that hexamethonium and stellate ganglionectomy

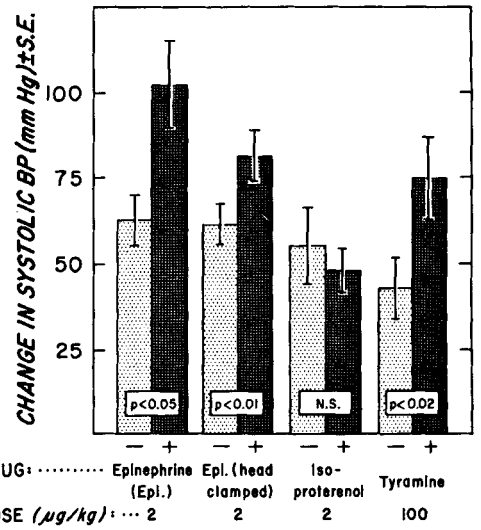

Fig. 9—Effect of pretreatment with ST-155 (8 mcg./Kg., i.v., 20 min.) on pressor responses to epinephrine and tyramine and vasodepressor response to isoproterenol and refer to ST-155.

lowered blood pressure to the extent that it could not be lowered further by ST-155 (Figs. 3 and 8). However, it must be emphasized that the "breakthrough" depressor responses to ST-155 after hexamethonium, *etc.*, were quantitatively minimal compared with the depressor activity seen in normal animals. It may be significant that whenever appreciable hypotension was seen, bradycardia was invariably present. Thus, it may well be that hypotension occurs as a result of a centrally mediated (reflex?) reduction in cardiac output (*cf.* 13). It is of interest to note that ST-155 appears to exert no bradycardia in the isolated spontaneously beating rabbit heart (Langenorff preparation) in doses of up to 100 mcg. (15).

Other studies in animals have shown that ST-155-induced hypotension is abolished by β-adrenergic blockade and by pentolinium (18), which is in agreement with the authors' interpretation of the action of ST-155 after hexamethonium or bretylium. Release of catecholamines by ST-155 would not seem to be involved since no change in cardiac norepinephrine occurs following high doses of ST-155 (1, 14). The sympathomimetic actions of ST-155 are abolished by tolazoline, phenoxybenzamine,[2] or phentolamine, but not by reserpine pretreatment (14, 17). It is thus likely that ST-155 has at least two components of action: an apparently "direct" musculotropic (α-adrenergic) action on blood vessels and nictitating membrane (1, 14, 17) and a centrally mediated bradycardia seemingly involving only the cardiac sympathetics.

The present studies pose a pertinent question of whether these findings can be extrapolated to explain the mechanism of hypotensive action of ST-155 in man. Significant reduction of cardiac output and heart rate was observed in a group of patients with essential hypertension receiving total doses of 150 to 300 mcg. of ST-155 intravenously (3). In these studies no appreciable change in peripheral

[2] Dibenzyline, Smith Kline and French Labs., Philadelphia, Pa.

resistance was noted. There have been reports of hypovolemia (7) and of redistribution of blood (*e.g.*, cutaneous vasconstriction) although these latter effects appear to be due to the vasoconstrictor action of ST-155 (16).

It cannot be stated at present if or how the supersensitization to the pressor actions of epinephrine and tyramine are related to the reflex hypotension or bradycardia. Moreover, that central inhibition of cardiac sympathetic nerve fibers by ST-155 does indeed occur must also remain an unresolved postulate until direct studies are made to test this hypothesis and to correlate this with the hypotensive action of ST-155. However, the authors of a related study which appeared shortly after the present investigation was completed, concluded that ST-155 owed its hypotensive action to a central inhibition of "medullary vasomotor and accelerator centers" (19).

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Keyphrases

Hypotensive activity—tolazoline analog
2-(2,6-Dichlorophenylamino)-2-imidazoline HCl (ST-155)—hypotensive activity
Pressor, depressor activity—ST-155
Cardiovascular activity—ST-155
Central mediation—ST-155 activity

Molecular Sorption on Ion-Exchange Resins: Studies with Coumarin

By R. S. HEGDE, M. J. MEHTA, R. A. BHATT, D. J. PATEL, and S. L. BAFNA

The sorption equilibrium of coumarin on ion-exchange resins of different degree of cross-linking, ionogenic groups, and counter ions in water and aqueous methanols has been studied and the results are discussed.

EARLIER, (1–3) the sorption and separation of coumarins on ion-exchange resins has been reported. This paper covers the study of the sorption equilibrium of coumarin on ion-exchange resins of different degrees of cross-linking, ionogenic groups, and counter ions in water and aqueous methanols at room temperature (~30°).

EXPERIMENTAL

Resins—(*a*) Styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins[1] of relative degree of cross-linking (% nominal divinylbenzene content) as 4, 8, and 12 (further referred to as X4, X8, and X12) of −100, +200 mesh; the resins were conditioned, regenerated, and moisture content and capacity were estimated (4, 5).

The different ionic forms of the resins were obtained by passing an excess of the salt or hydroxide solution through the resin bed. The resin was then washed, filtered, air dried, moisture content determined, and the capacity of the air-dried form of the resin calculated from the capacity of the oven-dried resin in the hydrogen form.

(*b*) Carboxylic acid cation-exchange resins[2] of relative degree of cross-linking (% nominal divinylbenzene content) X = 2.5, 5, 10, and 15 (further referred to as resin CX 2.5, CX 5, CX 10, and CX 15) of −40, +60 mesh; these are presumably based on acrylic acid divinylbenzene copolymers. The resins were conditioned, regenerated, and the moisture content and capacity were estimated as given before (5). The ionic forms of the resins were obtained by passing an excess of the corresponding hydroxide solution through the resin bed. The resins were then washed free of alkali, filtered, air dried, and the moisture content was determined. The capacity of the resins in different forms was calculated from the capacity of the oven-dried resin in the hydrogen form.

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[1] Dowex 50W, Dow Chemical Co., Midland, Mich.

[2] Zeo-karb 226, Permutit Co., London, England.