2,6-Dichlorobenzylidene Amino Guanidine Acetate (Wy-8678). A New Hypotensive Agent

Several useful hypotensive (i.e., antihypertensive) compounds have been introduced in the recent past. However, no substance has as yet satisfied all criteria for effectiveness and absence of untoward actions. Therefore, the search for more efficacious and safer drugs continues. Wy-8678 (2,6-dichlorobenzylidene amino guanidine acetate) represents a new class of hypotensive agents. It bears structural similarities to two other effective drugs, i.e., guanethidine and St 155 [Catapres, 2-(2,6-dichlorophenylamino-2-imidazoline hydrochloride)]. The present report compares the hypotensive activity of Wy-8678 and other substances in two widely used experimental models of hypertension. Although many substances are capable of lowering blood pressure in anesthetized animals only a few retain this action in conscious animals.

Methods. Female Sprague-Dawley rats were rendered hypertensive by the application of a Figure-8 ligature to the left kidney and contralateral nephrectomy as previously described[1,2]. Systolic blood pressure was recorded indirectly from the tail, using an occluding and sensing cuff. The latter (Decker Corp., Bala-Cynwyd, Pa.) detected the change in tail volume that occurred with each heart beat. Drugs were administered orally via stomach tube and pressures recorded prior to dosing and at 1.5, 4 and sometimes 24 h thereafter.

Dogs were rendered hypertensive in a 3-stage procedure, each performed under pentobarbital anesthesia (35 mg/kg). The carotid artery bifurcations were exposed by 2 incisions and the sinuses denervated by stripping the adventitia in the region. The right renal artery was exposed by a flank incision and constricted by a silver Goldblatt clamp. Finally, the left kidney was removed. Blood pressure was recorded indirectly from the tail with an occluding cuff and a condenser microphone by the method of PRIOLI and WINBURY[3]. Drugs were administered i.p. or orally. Data are reported as means ± standard errors.

Results. Several doses of Wy-8678 and other clinically useful hypotensive drugs were administered via stomach tube to unanesthetized hypertensive rats. Table I lists maximum changes in pressure that were encountered at any of the 3 observation periods regardless of time of occurrence. These peaks were usually observed at 1.5 h with Wy-8678, St 155 (Catapres), hydralazine and at 4 h with reserpine, guanethidine and methyldopa. It is evident that Wy-8678 is more potent on a weight basis than reserpine, guanethidine and methyldopa but less so than St 155 or hydralazine. On the other hand, Wy-8678 produced as great a response as St 155 and a somewhat smaller one than that obtained with the other substances but, nevertheless, the response to Wy-8678 amounted to a very large drop in pressure.

In some rats the peripheral pulse became undetectable at higher dosage levels of Wy-8678 and St 155 making pressure determinations impossible. This problem was encountered with the 4 mg/kg dose of Wy-8678 in that pressure could be read in only 8 of the 12 rats used. Had pressure been recorded directly greater responses would, in all probability, have been observed at higher doses.

Wy-8678 was administered to 4 groups of hypertensive dogs, i.e., i.p. in doses of 0.5, 1 and 2.5 mg/kg and orally at 5 mg/kg and pressure recorded at 2 and 4 h. All doses lowered blood pressure substantially but a clear dose-response relationship was not observed at the range used (Table II). Heart rate fell dramatically in all animals. St 155 produced results that were similar to those obtained with Wy-8678. Guanethidine did not lower pressure over the usual 4 h observation period. Therefore, a second dose was given in the evening. Pressure was considerably reduced by the next morning. Heart rate was not recorded in this series of experiments but was decreased by a mean of 16 beats/min in a previous group of 4 dogs.

Discussion. Although many effective hypotensive agents are currently available each of these substances is very far from the ideal and possesses troublesome side effects. Wy-8678 is a member of a newly studied chemical series possessing hypotensive actions. The present report examined the compound in 2 experimental models of hypertension. Wy-8678 lowered pressure in a dose-related manner when given orally to hypertensive rats. The degree of hypotensive activ-

Table I. Effects of Wy-8678 and other hypotensive agents on systolic blood pressure of hypertensive rats

	Dose (mg/kg)	No. of rats	Blood pressure (mm Hg) Control	Change
St 155	0.01	4	183 ± 10	−17 ± 3
	0.025	4	185 ± 12	−31 ± 6
	0.05	14	185 ± 7	−43 ± 7
	0.1	8	190 ± 8	−55 ± 10
Hydralazine	1	12	184 ± 5	−62 ± 9
	2.5	4	181 ± 6	−96 ± 5
Wy-8678	1	12	174 ± 5	−29 ± 7
	2	11	183 ± 8	−34 ± 9
	3	11	185 ± 7	−54 ± 8
	4	8	183 ± 7	−43 ± 5
Reserpine	2.5	8	167 ± 9	−22 ± 8
	5	16	181 ± 4	−47 ± 7
	10	8	174 ± 7	−74 ± 9
Guanethidine	10	4	179 ± 17	−27 ± 5
	20	12	180 ± 5	−41 ± 6
	40	8	188 ± 11	−64 ± 13
Methyldopa	50	8	168 ± 5	−42 ± 5
	75	4	198 ± 19	−83 ± 16
	100	4	164 ± 4	−67 ± 10

Table II. Effects of Wy-8678, St 155 and guanethidine on blood pressure and heart rate in hypertensive dogs

	Dose (mg/kg)	Route	No. dogs	Blood pressure (mm Hg) Control	Change	Heart rate (beats/min) Control	Change
Wy-8678	0.5	i.p.	4	204/131 ± 8/6	−28/−21 ± 11/9	102 ± 6	−50 ± 12
	1	i.p.	4	204/137 ± 10/9	−31/−17 ± 6/8	111 ± 10	−65 ± 7
	2.5	i.p.	4	190/135 ± 7/8	−31/−32 ± 5/9	92 ± 9	−45 ± 7
	5	orally	5	202/127 ± 9/5	−26/−34 ± 9/11	103 ± 7	−55 ± 9
St 155	0.025	orally	4	211/140 ± 13/9	−39/−35 ± 6/5	110 ± 17	−45 ± 14
Guanethidine	5 (twice)	orally	8	203/132 ± 9/7	−24/−20 ± 6/5	–	–

Changes refer to maximum decreases in pressure regardless of time of occurrence.

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ity attainable with this compound was somewhat less than that observed with several other agents, possibly because of the use of the indirect recording method. On the other hand, the compound was active at lower doses than reserpine, guanethidine or methyldopa, the 3 clinically most widely used substances. Wy-8678 also lowered pressure substantially in hypertensive dogs. A marked decrease in heart rate was also observed. The dose of guanethidine used was the maximum tolerated in these animals.

The above findings clearly indicate that Wy-8678 shows promise as a potentially useful therapeutic agent. A recent report showed that the compound also reduces pressure in anesthetized animals after an initial pressor phase[4]. The mechanisms responsible for the hypotensive action are currently under investigation. Preliminary experiments suggest that inhibition of sympathetic tone is a major feature of the compound[5].

Zusammenfassung. Bei unanästhesierten Ratten und Hunden wurden die antihypertensiven Eigenschaften von 2,6-Dichlorobenzyliden-Aminoguanidin-Azetat (WY 8678), welches zu einer neuen Reihe von aktiven Substanzen gehört, sowie noch einige andere klinisch wirksame Substanzen an zwei experimentellen Modellen für Hypertension geprüft und dabei eine wesentliche Blutdrucksenkung festgestellt.

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[5] St 155 was kindly supplied by Boehringer Ingelheim GmbH, guanethidine and hydralazine by Ciba Pharmaceutical Co., and methyldopa by Merck, Sharp & Dohme.

Electron Microscopic Observations on the Granular Vesicles in the Ciliary Ganglion of the Rat

On the basis of fluorescent microscopical studies, adrenergic nerve terminals have been postulated to exist in the ciliary ganglion of the cat, the goat, and the rat[1–3]. In addition, similar structures have been observed in the avian ciliary ganglion[4]. However, recent reports on electron microscopy fail to mention the existence of granular or 'dense core' vesicles in the avian ciliary ganglion[5–9]. Small granular vesicles (about 900 Å in diameter) have been presumed to store biogenic amines, which take part in the transmission of impulses in the nervous tissue, while larger granular vesicles (about 2000 Å in diameter) are generally interpreted as neurosecretory ones[10,11]. The present study was designed to investigate the mode of distribution of granular vesicles in the ciliary ganglion of the rat, a subject not previously studied.

About 20 male and female albino rats of Sprague-Dawley strain were used. The animals were decapitated under light ether anaesthesia. The fresh ganglia were quickly removed and fixed by immersion in 3.5% glutaraldehyde solution for 1–2 h, followed by immersion in 1% osmium tetroxide solution for 1–2 h. Both fixatives were ice-cold and buffered to pH 7.2 with 0.1 *M* phosphate. After rinsing in the buffer solution, the specimens were dehydrated in the graded alcohol series and embedded in Epon. The ultrathin sections were stained with lead citrate, and for electron micrography a Philips EM-200 apparatus was used. About 10 rats were injected i.p. with reserpine (Serpasil®, Ciba), 1 mg daily, 3 doses altogether, which treatment depletes the catecholamines from all autonomic synapses including those in the ciliary ganglion, as was demonstrated by formaldehyde-induced fluorescence[3]. Both ciliary ganglia of the injected animals were treated equally as those of the control rats. No difference in the results were observed between the sexes in the present study.

In the ciliary ganglion of the rat, granular vesicles were observed in 2 different regions. Firstly, granular vesicles were found in some nerve endings and synapses surrounding the nerve cell bodies. In these synapses, which are mostly of axodendritic type, the granular vesicles were localized in the presynaptic or axonal part of the synapse (Figure 1). In addition to these apparently

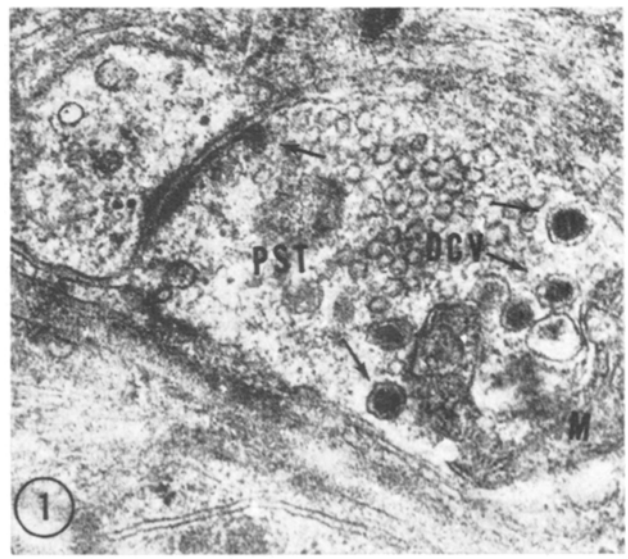

Fig. 1. Adrenergic synapse. Several granular vesicles in the presynaptic terminal. Ca. × 55,000.

Abbreviations used in the figures: A, Axon; DCV, Dense core vesicle = Granular vesicle; M, Mitochondrion; NC, Neuronal cytoplasm; NE, Nerve ending; NG, Neurosecretory granule; PST, Presynaptic terminal; S, Synapse.

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