Chlorisondamine Stabilization of Pressor Bioassays in Dogs

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Abstract □ Pretreatment of anesthetized dogs with chlorisondamine increases sensitivity to pressor responses of *d*-, *l*-, and *r*-epinephrines, and also the precision of bioassays by the USP XIV method. Over 1500 assays in such dogs showed the pressor potency of *d*-epinephrine to be 20% of the pressor potency of *l*-epinephrine.

Keyphrases □ Pressor bioassays, dogs—chlorisondamine stabilization □ Chlorisondamine effect—pressor response, *d*-, *l*-, *r*-epinephrine □ Epinephrine—relative potency, *d*, *l* isomers

Bioassays of *d*-, *l*- and racemic epinephrine solutions are still necessary since chemical procedures lack specificity. Details of the official procedure for assaying the pressor potency of *l*-epinephrine solution on anesthetized dogs, compared with the USP Epinephrine Reference Standard are spelled out in USP XIV (1, 2). Pressor potency bioassays are routinely performed in duplicate on different dogs. The variation in response between six dogs used in assaying the same sample may run as high as 50% (3). Which assay is likely to be correct? This is especially important when either duplicate shows substandard potency. Rising animal costs, coupled with the need to perform assays on an increasing number of samples, led to studies to reduce this variation between assays, to obtain greater reliability on a single test.

The basic problem arose in the use of hypertensive dogs, which showed low sensitivity to any injected pressor amine, with low assay values. A ganglion-blocking drug causing hypotension should produce a more suitable dog for assay. Chlorisondamine was introduced in 1956 for the treatment of severe hypertension in humans (4–15). It produced a heightened sensitivity to exogenous pressor substances in experimental animals (16, 17). Ganglionic blockade may play a role in maintaining steady basal blood pressure and uniform pressor responses by reducing cardiac output and increasing peripheral resistance (17–19). It was successfully used to obtain more uniform responses to norepinephrine and serotonin in assaying the antiserotonin-antihistaminic potency of a number of drugs. Without the use of chlorisondamine, assessment of antiserotonin activity would have been impossible because of the instability of serotonin response; ganglionic blockade produced a stable blood pressure floor which was unaltered by any of the antagonists studied (20).

EXPERIMENTAL

The reference solution used in these bioassays was prepared from USP XIV Reference Standard powder in accordance with directions therein. Reference solutions were freshly prepared and diluted at the time of use. The unknown solutions were prepared by dissolving synthetic racemic epinephrine hydrochloride in water and diluting to approximately the same concentration as that of the reference solution, at the time of bioassay.

Table I—100 Duplicate Pressor Bioassays on Dogs, Without Chlorisondamine, mg./ml.

I	II	Diff.	I	II	Diff.
3.55	4.17	0.62	3.96	4.45	0.49
3.61	4.17	0.56	3.97	4.24	0.27
3.62	3.83	0.21		4.55	0.58
3.65	3.99	0.34	3.98	4.27	0.29
	4.10	0.45		4.31	0.33
	4.14	0.49		4.31	0.33
3.66	4.04	0.38		4.31	0.33
3.67	4.11	0.44		4.32	0.34
3.68	4.12	0.44	3.99	4.06	0.07
	4.26	0.58		4.08	0.09
3.70	4.10	0.40		4.20	0.21
	4.35	0.65		4.23	0.24
3.74	3.80	0.06	4.00	4.06	0.06
	4.13	0.39	4.01	4.09	0.08
3.75	4.09	0.34		4.12	0.11
3.76	3.97	0.21	4.03	4.04	0.01
3.77	4.06	0.29		4.11	0.08
3.78	4.06	0.28	4.04	4.12	0.08
3.80	4.16	0.36		4.20	0.16
3.81	4.34	0.53		4.23	0.19
3.82	4.04	0.22	4.05	4.10	0.05
	4.36	0.54		4.14	0.09
3.83	3.92	0.09	4.06	4.13	0.07
	3.93	0.10		4.17	0.11
	4.11	0.28		4.32	0.26
	4.23	0.40	4.07	4.07	0
3.84	4.05	0.21		4.22	0.15
3.85	4.21	0.36		4.27	0.20
	4.29	0.44	4.08	4.08	0
	4.30	0.45		4.16	0.08
3.86	4.07	0.21		4.38	0.30
	4.43	0.57		4.39	0.31
	4.43	0.57	4.09	4.13	0.04
3.87	4.19	0.32		4.19	0.10
	4.38	0.41	4.10	4.12	0.02
	4.43	0.46		4.20	0.10
3.88	4.18	0.30	4.15	4.21	0.06
	4.26	0.38	4.17	4.19	0.02
3.90	3.95	0.05		4.27	0.10
3.91	3.95	0.04	4.19	4.21	0.02
	3.97	0.06		4.26	0.07
3.92	4.12	0.20	4.20	4.25	0.05
3.93	4.02	0.09	4.22	4.31	0.09
	4.47	0.54		4.32	0.10
3.94	4.35	0.41		4.41	0.19
3.95	4.04	0.09	4.23	4.34	0.11
	4.15	0.20		4.37	0.14
	4.34	0.39	4.32	4.47	0.15
3.96	4.08	0.12	4.39	4.43	0.04
	4.19	0.23	4.46	4.47	0.01

Dogs were given intravenous injections of 1 mg./kg. of chlorisondamine the afternoon of the day before use. The following morning they were anesthetized with chloralose and/or pentobarbital and atropinized until the vagal receptors were blocked. These treated dogs required less anesthesia, both initially and for maintenance. They were then given several injections of the Epinephrine Reference Standard solution and allowed to equilibrate. Systolic pressure should not exceed 150 mm. Hg; injection of 5 mcg. or less/kg. of the Reference Standard solution should produce an increase of about 50 mm. Hg; the pressor peak should be sus-

Table II—60 Duplicate Pressor Bioassays on Dogs, after Chlorisondamine

Bioassay, mg./ml.			Bioassay, mg./ml		
I	II	Diff.	I	II	Diff.
3.75	3.86	0.11	4.01	4.12	0.11
3.80	3.96	0.16		4.16	0.15
3.83	4.02	0.19		4.17	0.16
3.87	4.08	0.21	4.02	4.09	0.07
3.88	4.04	0.16		4.21	0.19
	4.04	0.16	4.03	4.08	0.05
3.89	4.04	0.15		4.19	0.16
3.90	4.00	0.10		4.26	0.23
	4.04	0.14	4.04	4.04	0
3.91	3.98	0.07		4.10	0.06
3.92	3.92	0		4.21	0.17
	4.11	0.19		4.22	0.18
3.93	3.95	0.02	4.05	4.16	0.11
	4.02	0.09		4.17	0.12
3.95	4.08	0.13	4.06	4.15	0.09
	4.12	0.17	4.08	4.24	0.16
	4.12	0.17	4.09	4.32	0.23
3.96	4.24	0.28	4.10	4.15	0.05
3.97	3.99	0.02	4.11	4.13	0.02
	4.19	0.22	4.13	4.20	0.07
	4.26	0.29	4.14	4.32	0.18
3.98	4.14	0.16	4.15	4.19	0.04
3.99	4.11	0.12	4.16	4.29	0.13
4.00	4.09	0.09	4.17	4.18	0.01
	4.11	0.11	4.18	4.29	0.11
	4.14	0.14	4.19	4.21	0.02
	4.19	0.19		4.24	0.05
	4.25	0.25	4.20	4.25	0.05
4.01	4.02	0.01	4.23	4.38	0.15
	4.10	0.09	4.27	4.28	0.01

tained through several heartbeats. Until such conditions are satisfied, additional small doses of chlorisondamine are injected slowly (0.2–0.5 mg./kg. over 2 to 5 min.).

In these ganglion-blocked dogs, the unique pressor response is characterized by a steady rise in both systolic and diastolic pressures; no bimodal record; and a smooth decline to the base line with no depressor phase. The heart rate is slowed, and the time interval between injection of the pressor material and return of the systolic pressure to the preadministration level required several minutes longer than in untreated dogs. Sensitivity to pressor substances tends to increase, so that the injection of 0.1 mcg./kg. of Reference Epinephrine solution often produces a rise of 50 mm. Hg in systolic pressure.

Prior to the use of chlorisondamine, the standard deviation of assays was 6% of the stated potency; even more disturbing was the observation that variation between dogs in duplicate assays was large enough to require repeating one-third of the assays. In the most recent 500 assays on 150 dogs receiving chlorisondamine, the standard deviation was reduced to 3.3% of the stated potency. Reports on 100 duplicate bioassays in which chlorisondamine was not used are shown in Table I. The lower assay response is shown in the first column, the re-assay in the second column, and the difference between these duplicate assays in the third column. The line separates those assays in which the lower result fell below the acceptable potency of 3.90 mg./ml. This included 38 original assays, and the differences between duplicate tests tended to be larger than in the assays at the higher dosage response levels.

Responses to 60 duplicate assays on dogs after the administration of chlorisondamine are listed in Table II. Of these, only seven original assays fell below 3.90 mg./ml., and differences between duplicate assays are smaller. The ranges of assay values by group intervals are condensed in Table III. In the range of original values between 3.90 and 3.99 mg./ml., there were 24 re-assays in the dogs without chlorisondamine, and the increments were between 0.04 and 0.58; in 16 dogs in this response level receiving chlorisondamine, the increments ranged from 0 to 0.29 mg./ml. For the entire set of 100 re-assays without chlorisondamine, the mean range was 0.24 mg./ml., with a standard error (*SE*) of 0.02 mg./ml.; 60 duplicate assays in dogs which received chlorisondamine showed a

Table III—Differences in Duplicate Pressor Potency on Dogs

Range of Lowest Assay Values, mg./ml.	Increases in Re-assay Same Sample Without Chlorisondamine		With Chlorisondamine	
	No.	Range, mg./ml.	No.	Range, mg./ml
3.50–3.79	18	0.06–0.65	1	0.11
3.80–3.89	20	0.09–0.57	6	0.15–0.21
3.90–3.99	24	0.04–0.58	16	0 –0.29
4.00–4.09	22	0 –0.31	24	0 –0.25
4.10–4.19	7	0.02–0.10	10	0.01–0.18
4.20–4.49	9	0.01–0.19	3	0.01–0.15
$\bar{X} \pm SE$	100	0.24±0.02	60	0.12±0.01

mean range of 0.11 mg./ml., with an *SE* of 0.01 mg./ml. The difference between these mean values is statistically significant.

DISCUSSION

The original studies by Cushny suggested that *d*-epinephrine had one-twelfth to one-fifteenth the potency of *l*-epinephrine; however, the *d* form was always contaminated with some of the *l* isomer (personal communication). This relative potency has been included in various textbooks of pharmacology (21). Other investigators have indicated that the pressor potency of *d*-epinephrine is about 4% of that of *l*-epinephrine. Since the racemic form contains equal parts of the *l* and the *d* isomers, the expected pressor potency would be about 52% of that shown by pure *l*-epinephrine. In pressor studies on over fifteen hundred chlorisondamine-treated dogs, the potency of racemic epinephrine was found to be 60 ± 2% of that of the *l* form. This would suggest that, under these conditions, *d*-epinephrine has a pressor potency of 20% of that shown by *l*-epinephrine. Since the *d* form contains varying amounts of the *l* isomer, it has not been practical to make direct comparisons.

SUMMARY

The variations in pressor potency of *d*-, *l*- and *dl*-epinephrines, when assayed by the USP XIV pressor method on anesthetized dogs, are significantly reduced by pretreatment with chlorisondamine.

Over 1500 comparative assays in such animals suggests that the pressor potency of *d*-epinephrine is of the order of 20% of the potency of *l*-epinephrine.

The use of chlorisondamine reduces the variability, the time required, and expense of bioassays of these pressor products.

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Stereospecific Hydrogenations IV: Palladium-on-Poly-*S*-Valine and Palladium-on-Poly-*S*-Leucine

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Abstract □ Studies concerning the influence of helical conformation on the enantioselective hydrogenations catalyzed by palladium-on-poly-*S*-amino acids are described. Poly-*S*-valine which does not normally form a helical conformation and poly-*S*-leucine which forms a right-handed helix were chosen as carriers for this study. Hydrogenations of α-methylcinnamic acid and α-acetamidocinnamic acid using palladium-on-poly-*S*-valine produced predominantly *S*(+)-dihydro-α-methylcinnamic acid and R(+)-phenylalanine (after hydrolysis), respectively. The hydrogenations catalyzed by palladium-on-poly-*S*-leucine produced predominantly R(−)-dihydro-α-methylcinnamic acid and *S*(−)-phenylalanine (after hydrolysis), respectively. These results indicate that the helical conformation of the polyamino acid carriers does influence the asymmetric induction observed. The asymmetric induction arising from the chirality of the screw sense of the polyamino acid appears to have a stronger influence on the configuration of the products than does the asymmetric induction arising from the configurations of the amino acid residues. The asymmetric induction arising from the helical conformation appears to mask any influence arising from the asymmetric carbon atoms of the amino acid residues.

Keyphrases □ Palladium-on-poly-*S*-valine—α-methylcinnamic and α-acetamidocinnamic acid hydrogenation □ Palladium-on-poly-*S*-leucine—α-methylcinnamic and α-acetamidocinnamic acid hydrogenation □ Asymmetric induction—polyamino acid, helical conformation.

Stereospecific hydrogenations using palladium-on-poly-*S*-leucine, palladium-on-poly-γ-benzyl-*S*-glutamate and palladium-on-poly-β-benzyl-*S*-aspartate were described in earlier papers (1, 2). The substrates used in these hydrogenations were α-methylcinnamic acid and α-acetamidocinnamic acid, both of which produce asymmetric carbon atoms on hydrogenation.

Predominantly R(−)dihydro-α-methylcinnamic acid was formed when α-methylcinnamic acid was hydrogenated using either palladium-on-poly-*S*-leucine or palladium-on-poly-γ-benzyl-*S*-glutamate. *S*(−)-Phenylalanine was formed when α-acetamidocinnamic acid was hydrogenated using these same catalysts and the hydrogenation product hydrolyzed with dilute aqueous hydrochloric acid. Catalysts prepared from poly-β-benzyl-*S*-aspartate induced the formation of predominantly *S*(−)-dihydro-α-methylcinnamic acid and predominantly R(−)-phenylalanine, respectively, when the same substrates were used. Since both poly-*S*-leucine and poly-γ-benzyl-*S*-glutamate form stable helices having a right-handed screw sense and poly-β-benzyl-*S*-aspartate forms an anomalous left-handed helix (3–6), the helical sense of the poly-*S*-amino acid carrier must influence the asymmetric induction observed.

The present paper describes studies made to compare the influence of the chirality of the secondary structure (helix) *versus* the chirality arising from the asymmetric carbon atoms of the *S*-amino acid residues on the steric course of the enantioselective hydrogenations.

The carrier polyamino acids chosen for these studies were poly-*S*-valine and poly-*S*-leucine. As noted above, poly-*S*-leucine possesses a right-handed helix. Poly-*S*-valine, a lower homolog of poly-*S*-leucine differing from poly-*S*-leucine by one methylene group per amino acid residue, does not normally form a helical conformation, but forms a random β structure because of steric hindrance (7).

EXPERIMENTAL[1]

Reagents—*N*-Carbobenzyloxy-*S*-valine (Nutritional Biochemicals), α-methylcinnamic acid (Aldrich), α-acetamidocinnamic acid (Aldrich), leucine (Mann Biochemical Corp.)., and carbobenzyloxy chloride (Nutritional Biochemicals).

N-Carboxy-S-valine Anhydride (I)—This compound was prepared according to the Leuchs procedure (8). Twelve grams (0.1 mole) of glass-distilled thionyl chloride were added to 12.5 g. (0.05 mole) of *N*-carbobenzyloxy-*S*-valine. The mixture was warmed gently to 40° until evolution of gas diminished and heated on a

[1] All temperatures are uncorrected. Elemental analyses were determined using a Hewlett-Packard model 185 C, H, and N analyzer. Optical rotation measurements were made using a Rhudolph model 200S polarimeter. A Perkin-Elmer Infracord model 137B spectrophotometer was used to obtain the IR spectra.