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SPECIFIC PRESSOR ACTIVITY OF ANGIOTENSINS I AND II

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It is generally accepted that the biological activity of 5-isoleucine- and 5-valine-angiotensin II is equal even when aspartic acid in position 1 is substituted by asparagine (1). Angiotensin I has approx. one third the pressor activity of angiotensin II (1). However, a survey of literature reveals certain inconsistencies (2-10) (Table 1).

We synthesized Asp¹-Ile⁵-angiotensin I and II by the classical procedures (11), and purified them by SE-Sephadex column chromatography (12). We also purified Asn¹-Val⁵-angiotensin II (Ciba Lot-171671). Purity of these angiotensins was checked after dansylation (13). They showed single spots on a thin-layer chromatogram of Silica gel H. Their weights were determined by optical density of tyrosine (OD 275) on approx. 10 mg of the pure samples.

A four-point assay was performed on various combinations of angiotensins I or II in 6 rats, injecting each with four of low and high doses. Female rats of the Donryu strain, weighing 200 ± 20 g, were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and treated with pentolinium tartrate (5 mg/kg, i.v.). The femoral artery and vein were cannulated for blood pressure registration and intravenous injection respectively.

Angiotensins were dissolved in 0.3 ml of 0.9% NaCl containing 0.001% (v/v) of Tween 20. Low and high doses were 1 or 2 and 10 or 20 ng respectively. These doses had been determined in preliminary experiments, in which 0.2 to 40 ng of angiotensins were used to examine linearity and parallelism in dose-response curves of two samples.

The data were analyzed statistically by the routine 2×2 parallel line assay (14) (Table 2). We found that the pressor activity of Asn¹-Val⁵-angiotensin II and Asp¹-Ile⁵-angiotensin II was not equal in the rat, suggesting that native angiotensin (13) is more potent.

TABLE 1. Specific pressor activity of angiotensins I and II

Angiotensin	Sp. activity in weight (%)	Assay animal	Reference
Asp ¹ -Ile ⁶ -Ang II	100	rat	Gross & Turrian, 1960 (2)
Asp ¹ -Val ³ -Ang II	100		
Asn ¹ -Ile ⁶ -Ang II	100		
Asn ¹ -Val ³ -Ang II	100		
Asp ¹ -Val ³ -Ang II	100	rat	Paiva & Paiva, 1960 (3, 4)
Asn ¹ -Val ³ -Ang II	53 ± 4		
Asn ¹ -Ile ⁶ -Ang II	28 ± 4		
Asp ¹ -Ile ⁶ -Ang II	100	rat	Bumpus et al., 1961 (5)
Asp ¹ -Ile ⁶ -Ang I	86		
Asn ¹ -Ile ⁶ -Ang II	85		
Asn ¹ -Val ³ -Ang II	100	rat	Guttmann, 1961 (6)
Asp ¹ -Val ³ -Ang I	89		
Asp ¹ -Ile ⁶ -Ang II	100	rat	Page & Bumpus, 1961 (1)
Asp ¹ -Val ³ -Ang II	100		
Asn ¹ -Ile ⁶ -Ang II	100		
Asn ¹ -Val ³ -Ang II	100		
Asp ¹ -Ile ⁶ -Ang I	52		
Asp ¹ -Ile ⁶ -Ang II	100	cat	Helmer, 1964 (7)
Asp ¹ -Val ³ -Ang II	100		
Asn ¹ -Val ³ -Ang II	100		
Asp ¹ -Ile ⁶ -Ang II	100	rat	Gould, Skeggs & Kahn, 1966 (8)
Asn ¹ -Val ³ -Ang II	57		
Asp ¹ -Ile ⁶ -Ang I	60		
Asn ¹ -Val ³ -Ang II	100	rat	Boyd et al., 1969 (9)
Asn ¹ -Val ³ -Ang I	60		
Asn ¹ -Val ³ -Ang II	100	rat	Kreye & Gross, 1971 (10)
Asn ¹ -Val ³ -Ang I	58		
Asp ¹ -Ile ⁶ -Ang I	45		
Asp ¹ -Ile ⁶ -Ang II	100	rat	Arakawa, (personal communication)
Asp ¹ -Ile ⁶ -Ang I	55 (45-75)		

TABLE 2. Specific pressor activity of angiotensins in the rat by 4-point assay

T	S	PR _w	PR _M
Asp ¹ -Ile ⁶ -Ang I	Asn ¹ -Val ³ -Ang II (Ciba Lot-051640)	0.904 (0.845-0.968)	1.136 (1.062-1.217)
Asp ¹ -Ile ⁶ -Ang I	Asn ¹ -Val ³ -Ang II (Ciba Lot-171671)	0.716 (0.678-0.757)	0.900 (0.852-0.952)
Asp ¹ -Ile ⁶ -Ang II	Asn ¹ -Val ³ -Ang II (Ciba Lot-171671)	1.290 (1.235-1.347)	1.309 (1.254-1.367)
Asp ¹ -Ile ⁶ -Ang I	Asp ¹ -Ile ⁶ -Ang II	0.510 (0.488-0.532)	0.632 (0.605-0.659)
Asn ¹ -Val ³ -Ang II	Asn ¹ -Val ³ -Ang II (Ciba Lot-171671)	0.951 (0.893-1.014)	0.951 (0.893-1.014)

T : test material, S : standard material, PR_w : potency ratio on a wt. basis, PR_M : potency ratio on a molar basis. The 95% confidence interval is in parentheses.

The potency ratio of Asp¹-Ile⁵-angiotensin I relative to Asp¹-Ile⁵-angiotensin II was 0.51 on a weight basis, or 0.63 on a molar basis, which accords with previously reported data (1).

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MORPHINE SPECIFIC SEIZURE INDUCED IN POLY-D-LYSINE PRETREATED MICE AND SOME EVIDENCE SUGGESTING THE PRESENCE OF A NOVEL SITE FOR THE MORPHINE EFFECT

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While studying the pharmacological properties of a morphine derivative covalently bound to the macromolecules, it was noticed that the mice, after being intracranially pretreated with poly-D-lysine carrying the morphine derivative, responded to the s.c. injected dose of morphine in an unusual manner (1). A peculiar type of seizure, like the one inducible by strychnine, occurred and the animals died. The phenomenon was observed to similarly occur in mice pretreated with poly-D-lysine.

In the solution of physiological pH, the polymer exists as a strongly charged polycationic molecule and then, when such a molecule comes into contact with the cell membrane, the easily conceivable consequence would be that the functions of the cell would more or less be inevitably modified (2-7). From this viewpoint, the above described ob-