Summary of thin-layer chromatographic data obtained through 4 extraction procedures for potato tubers which were dipped by suction infiltration in various concentrations of N^6-benzyladenine (N6BA).

		Ammonia precipitation								Warm carbonate extraction							
Rf		Dark				Light				Dark				Light			
×		ppm N6BA				ppm N6BA				ppm N6BA				ppm N6BA			
100		0	250	500	1000	0	250	500	1000	0	250	500	1000	0	250	500	1000
100	–					–				–				–			–
										3		4					
											2						
80	–					–				–				–		4	3 –
														2	3		
					2			5		2		2	3				
		2[a]	4			3			5								
60	–			3	2	–	5	4	4	–				–		3	–
						1										4	1
		1	4	2			4					1			3		
										3			4	3	4		4
40	–					–				–				–	5		–
													4				
20	–					–				–				–			–
0	–					–				–				–			–

seem that in this case N^6-benzyladenine promotes the formation of glycoalkaloids in the dark and is even more influenced to do so in the light. Others have noticed the potentiation of a cytokinin effect by red light[10].

The non-ammonia precipitation extracts produced spot separations of several unidentified glycoalkaloids. The warm carbonate extraction usually consisted of 3 separations per treatment. The dark reaction promoted an intense, high Rf value spot at 500 ppm N^6-benzyladenine. Of the compounds extracted by hot water, the 250 ppm N^6-benzyladenine-dark treatment seemed most inhibitory to glycoalkaloid formation. The 1000 ppm N^6-benzyladenine-light treatment led to an increase in visual density of the highest Rf entity and the apparent origin of an additional compound. The individuals appearing in the ethyl acetate extraction were generally unchanged by either light or dark or N^6-benzyladenine treatments, excepting the 2 dense spots from light-treated potatoes receiving no N^6-benzyladenine treatment.

As indicated with the ammonia extractions, low concentrations of N^6-benzyladenine promote the formations of α-solanine and α-chaconine even in tubers stored in darkness. This situation was less apparent in extractions of unknown glycoalkaloids which would indicate an N^6-benzyladenine interference with solanidine formation and its incorporation into the glycoalkaloidal structure. The other unidentified glycoalkaloids demonstrate some differences in spot separation and intensity from dark to light treatments and when under the influence of N^6-benzyladenine. Perhaps the formation of the glycoside moieties in light and dark situations are also affected by this cytokinin.

Zusammenfassung. Behandlung der Sprossknollen mit Benzyladenin fördert die Glykoalkaloidbildung der Solanaceen. Je nach Extraktionsmethode können unidentifizierte Alkaloide nachgewiesen werden.

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Enhancement of the Blood Pressure Activity of VAL^5- and ILE^5-Angiotensin II by Sodium and Calcium Ions[1,2]

Enhancement by certain cations of several biological activities of Val^5-angiotensin II-Asp^1-β-amide (Asn^1, Val^5-A II) has previously been demonstrated. When the peptide is dissolved in hypertonic or isotonic NaCl solution rather than hypotonic solution or distilled water its dipsogenic, antidiuretic and natriuretic effects are enhanced when it is injected into the third brain ventricle in goats[3,4]. Similar studies include rat, guinea-pig, and bird pressor activities and stimulation of isolated rat uterus and guinea pig ileum[5–7]. Certain additional mono- and divalent cations were found to be effective in enhancing the rat blood pressure (bp) activity of Asn^1, Val^5-A II[6,7].

In the present study we investigate whether the effects of NaCl and $CaCl_2$ are restricted to the amide derivative of angiotensin II or whether they are also observed with the natural hormones, Asp^1,Val^5-angiotensin II (Val^5-A II) and Asp^1-Ile^5-angiotensin II (Ile^5-A II).

Methods. The rat bp assay was performed on nephrectomized rats as previously described[7], except that the

arterial catheter was attached to a Statham P23 AC transducer connected to a Grass Model 7 polygraph. The naturally occuring Val^5-A II obtained from Dr. B. RINIKER (Ciba-Geigy AG, Basel) and Dr. J. M. STEWART (University of Colorado Medical School, Denver) and Ile^5-A II (Calbiochem, lot 200873) were dissolved in NaCl or $CaCl_2$ solution for the Test Solutions and in distilled water for the Control Solutions as described[7]. Asn^1, Val^5-A II (Hypertensin®, Ciba) dissolved in 150 mM NaCl served as the standard. All solutions were prepared in cellulose nitrate tubes (Beckman No. 302235). The injection sequence was as follows: 5–10 Asn^1, Val^5-A II standard injections at equal and at increasing concentrations in order to test for reproducibility of the bp response and acceptable dose-response relationship, respectively; then Asn^1, Val^5-A II standard, hormone test solution, hormone control solution, Asn^1, Val^5-A II etc. The Asn^1, Val^5-A II standards contained 50, 100 and 150 ng peptide/ml 150 mM NaCl.

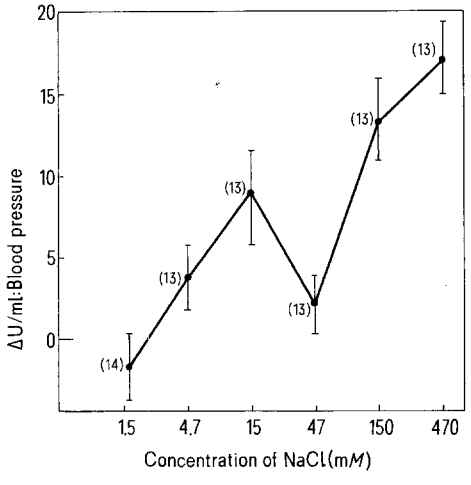
The recorded amplitudes of bp increase are expressed in terms of the Asn^1, Val^5-A II standards, minimizing possible errors due to changes of sensitivity in the test system in the course of the experiment. Because of the solvent dependence of the activities of Asn^1, Val^5-A II and Ile^5-A II (see below), it was necessary to denote the activity recorded with 100 ng Asn^1, Val^5-A II/ml 150 mM NaCl arbitrarily as 100 units/ml. Since each test solution was preceded or followed by a control solution (identical amount of Val^5-A II or Ile^5-A II dissolved in distilled water), Δ values can be given: Δ = activity per ml of test solution minus activity per ml of control solution. Paired samples were compared by a t-test, and different series of experiments by the nonparametrical Wilcoxon test. Each series was tested in 5 different rats.

Results. In the bp assay 3 rats had to be eliminated because of unacceptable dose-response relationship. With the remaining 24 rats a total of 190 determinations (test and control solutions) was obtained (7.9/rat). The bp increases observed with 50 ng or with 100 ng of Asn^1, Val^5-A II per ml of 150 mM NaCl i.v. at the beginning of the test were 17.9 $\pm$ 1.4 mm Hg (n 24) and 29.8 $\pm$ 1.7 mm Hg (n 24), respectively. At the end of the tests the responses to these two standards were 16.7 $\pm$ 1.6 mm Hg (n 24) and 29.9 $\pm$ 2.0 mm Hg (n 24).

Table I shows the effect of 150 mM NaCl on the rat bp response to Asn^1, Val^5-A II, Val^5-A II and Ile^5-A II. The effect of increasing concentrations of NaCl on the activity of the Ile^5-A II is shown in the Figure. In addition, the influence of 15 mM $CaCl_2$ on the bp response to Asn^1, Val^5-A II, Val^5-A II and Ile^5-A II is given in Table II.

Discussion. This investigation shows that the capability of NaCl to enhance the bp response in the nephrectomized rat is not restricted to the amide derivative of angiotensin II, but is also observed with the natural hormones Val^5-A II and Ile^5-A II. The effect of NaCl on the activity of natural hormones is somewhat less than the effect on the activity of the amide derivative (Table I); however, the pattern of the enhancement of activity over the range of 1.5 to 470 mM NaCl is essentially the same for Ile^5-A II (Figure) and Asn^1, Val^5-A II[7].

It was reported earlier that the activity of Asn^1, Val^5-A II was sensitive to $CaCl_2$. This divalent cation is more effective in enhancing the bp activity of Asn^1, Val^5-A II than any of the monovalent cations studied, including



Effect of increasing concentrations of NaCl on Ile^5-angiotensin II. The activities of 100 ng of Ile^5-A II per ml, dissolved and injected in NaCl solutions ranging from 1.5 to 470 mM, were measured and compared with the activities of the corresponding concentration of Ile^5-A II in water as a control. The ordinate gives the differences in bp activity between standardized test and control solutions, expressed in Δ values, where Δ = activity per ml of the Ile^5-A II test solution minus activity per ml of the control solution ($\bar{x} \pm$ S.E.). The NaCl concentrations are plotted on the abscissa.

[1] Supported by USPHS Grant No. AM-13567 and AM-18399.

[2] Abbreviations used are: A II, angiotensin II; bp, blood pressure; n, number of experiments; $\bar{x}$, mean value; S.E., standard error; p, level of significance; NS, not significant.

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Table I. Effect of 150 mM NaCl on the pressor activity of Asn^1, Val^5-angiotensin II; Val^5- and Ile^5-angiotensin II

Test solution	n	Δ	P		Intergroup p	
Asn^1, Val^5-A II	13	21.0 $\pm$ 2.0	> 0.001			
Val^5-A II	28	11.1 $\pm$ 2.6	> 0.001	NS	> 0.01	> 0.05
Ile^5-A II	13	13.3 $\pm$ 2.5	> 0.001			

The test solutions consisted of 100 ng of each of the 3 peptides per ml, and Δ represents the activity of the test solution minus the activity of the control solution in units per ml ($\bar{x} \pm$ S.E.).

Table II. Effect of 15 mM $CaCl_2$ on the pressor activity of Asn^1, Val^5-angiotensin II; Val^5- and Ile^5-angiotensin II[a]

Test solution	n	Δ	P		Intergroup p	
Asn^1, Val^5-A II	12	21.3 $\pm$ 2.2[b]	> 0.001			
Val^5-A II	25	3.7 $\pm$ 2.9	NS	> 0.001	> 0.001	
Ile^5-A II	16	3.5 $\pm$ 1.7	NS			

[a] For explanation see Table I. [b] Value taken from reference[7].

Na^+[7]. We found in this study that 15 m*M* $CaCl_2$ did not significantly increase the activity of either the Val[5]-A II or Ile[5]-A II (Table II) Previous caution that our results could be influenced by the intrinsic activity of Ca^{++} in modifying the bp activity appears to be unfounded, at least at the $CaCl_2$ concentration used. Moreover, the fact that $CaCl_2$ enhanced the bp activity of Asn[1], Val[5]-A II but failed to significantly enhance the bp activity of the Val[5]-A II and Ile[5]-A II, is further evidence against the possibility that the peptides reach the vascular receptors in a 'bolus', not unlike the results of an earlier investigation[7].

This investigation shows that the degree of enhancement of activity of a given angiotensin II peptide varies with the ion present. In addition it is apparent that the activity of the naturally occuring angiotensins is affected by a given ion in a different manner than the amide derivative. These results lend further support to the hypothesis of a selective interaction of each ion with the hormone or its analogs leading to an alteration of the equilibrium of the preferred conformation which is detected by changes in the bp activity[5,8].

Zusammenfassung. Nachweis, dass die Blutdruckaktivität von Val[5]-, Ile[5]- und Asn[1],Val[5]-Angiotensin II durch NaCl gesteigert wird. Die Aktivität der natürlichen Hormone wird jedoch von $CaCl_2$ nicht signifikant erhöht, obwohl das Ion die Aktivität von Asn[1],Val[5]-Angiotensin II stark vermehrt.

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Morphological Aspects of the Functional Synchronization of Supraoptic Nucleus Neurons

It is well-known that the neurons of the supraoptic nucleus are involved in the synthesis, transport and liberation of the neurohormones ADH and oxitocin[1,2]. The secretory activity of the hypothalamic-neurohipophyseal system in vertebrates has been studied in both normal and experimental conditions, the latter producing hypo- and hyperfunction[3]. To evaluate the functional state of the neurosecretory neurons, the nuclear and nucleolar volumes and the development of Nissl substance were used as parameters by ZAMBRANO and MORDOH[4]. They demonstrated periods of asynchronic secretory activity in neurosecretory neurons of normal rats, during which different zones in the supraoptic nucleus could vary their functional state, although the degree of activity within each zone was maintained constant. This functional zonal asynchrony is maintained in animals stimulated by dehydration, although a certain degree of synchronization of all neurosecretory neurons during rehydration can be observed[5,6]. In our study we wish to analyze some ultrastructural aspects of the neurosecretory neurons in the supraoptic nucleus, suggesting the existence of functional coordination and synchronization processes among these neurons.

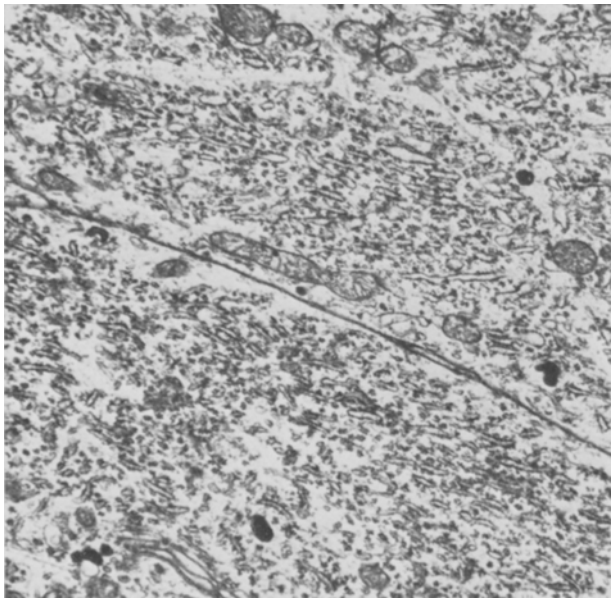

Fig. 1. Wide zone of apposition without interposition of a glial barrier in 2 neurosecretory neuron somas. Numerous granular endoplasmic reticulum cisterns are seen in the marginal cytoplasm of these neurons. ×12.000.

Materials and methods. The ultrastructure of hypothalamic supraoptic nucleus in both sexes of Wistar rats was studied. The rats were fixed by perfusion of 3% glutaraldehyde and the hypothalamic blocks were postfixed in 2% osmium tetroxide. Both fixatives were buffered at pH 7.4 in 0.12 *M* phosphate buffer. The hypothalamic blocks were embedded in Durcupan (Fluka) as usual and stained with 1% aqueous uranyl-acetate and then lead citrate.

Results. The present ultra-structural study of normal rat supraoptic nucleus neurons is in accord with studies previously described[7,8]. Briefly, the neurons have a large nucleus with a prominent nucleolus. The cytoplasm presents 2 well-defined regions: a perinuclear region with a very well developed Golgi apparatus, containing lysosomes and neurosecretory vesicles; and a marginal cytoplasm, very rich in free ribosomes and cisterns of granular endoplasmic reticulum.

Different functional states of the neurons of the supraoptic nucleus are reflected by the variations seen in the

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