

Phyton (Austria)	Vol. 22	Fasc. 1	143—148	15. 2. 1982
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## The Interaction of the Excitatory Substance with Auxin in *Elodea*

By

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Received June 15, 1981

Key words: excitatory substance, 1-methylhistidine, auxin, *Elodea*

### Summary

UMRATH K. & WATANABE S. 1982. The interaction of the excitatory substance with auxin in *Elodea*. — *Phyton (Austria)* 22 (1) 143—148. — English with German summary.

In the midrib of leaves of *Elodea canadensis* MICHX. the percentage of cells with systrophe of the chloroplasts and of cells without protoplasmic streaming is augmented in solutions of 0,1 mM IAA and of  $10^{-3}$  to  $10^{-5}$  mM 1-methylhistidine on the 2. and 3. day of the experiment. In solutions in which these two substances are combined these effects are diminished or abolished.

The velocity of protoplasmic streaming on the 4. and 5. day of the experiments is diminished in  $10^{-2}$  to  $10^{-4}$  mM 1-methylhistidine and to a small extent also in 0,1 mM IAA. In solutions containing both these substances the velocities are higher than in solutions of either of the substances alone.

The results suggest that unfavourable effects of 1-methylhistidine, suggested as excitatory substance in *Hydrocharitaceae*, and of IAA in certain concentrations are reduced or abolished in mixture of both substances, as well as a certain quantitative relation between excitatory substance and auxin is favourable.

### Zusammenfassung

UMRATH K. & WATANABE S. 1982. Die Wechselwirkung von Erregungssubstanz mit Auxin bei *Elodea*. — *Phyton (Austria)* 22 (1): 143—148. Englisch mit deutscher Zusammenfassung.

In Lösungen von 0,1 mM IAA bzw. von  $10^{-3}$  bis  $10^{-5}$  mM 1-Methylhistidin sind am 2. und 3. Tag des Versuches in der Mittelrippe von Blättern von *Elodea*

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*canadensis* MICHX. der Prozentsatz der Zellen mit Systrophe der Chloroplasten und derjenigen der Zellen ohne Protoplasmaströmung erhöht. In Lösungen, in denen diese beiden Substanzen kombiniert sind, sind diese Effekte verringert oder aufgehoben.

Die Geschwindigkeit der Protoplasmaströmung am 4. und 5. Tag der Versuche wird durch  $10^{-2}$  bis  $10^{-4}$  mM 1-Methylhistidin verringert und geringfügig auch durch 0,1 mM IAA. In Lösungen beider Substanzen ist die Strömungsgeschwindigkeit höher als in Lösungen jeder der beiden Substanzen allein.

Die Ergebnisse zeigen, daß sich ungünstige Wirkungen von 1-Methylhistidin, nach FITTING die Erregungssubstanz der Hydrocharitaceen, und von IAA in Mischungen beider Substanzen aufheben und beide Substanzen, in bestimmtem Verhältnis geboten, fördernd wirken können.

### Introduction

FITTING 1925 found that extracts of *Vallisneria* and of *Elodea* elicit protoplasmic streaming in both plants. Further investigations of FITTING 1936, 1937 indicated that 1-methylhistidine is the exciting substance in the extracts. UMRATH 1930 found that the excitatory substance of plants of different families are chemically different, but that all plants of one family or of one subfamily have the same excitatory substance. As protoplasmic streaming in the *Hydrocharitaceae* is elicited by light and other stimuli it can be taken as a sign of the excitation process and 1-methylhistidine as the excitatory substance.

It was found in not yet published investigations of *Elodea* in the institute for plant physiology at Graz, that some substances, including 1-methylhistidine and 3-indolylacetic acid (IAA), have unfavourable effects in higher concentrations. We wanted to clear the question if the unfavourable effects result from a high and uncompensated concentration of auxin on the one hand and of the excitatory substance on the other hand. For this purpose we investigated IAA and 1-methylhistidine alone as well as combinations of both.

### Material and Methods

*Elodea canadensis* MICHX. was grown in an aquarium with tap water. It stood on the side of the glasses with the experimental plants under the same light conditions.

The experimental plants were in glass vessels of 1 l in volume, 18,5 cm high, with a base of  $12,2 \times 5,7$  cm. Each vessel contained 4 shoots of *Elodea*, 20 cm in length with their apical ends intact. Two vessels contained tap water, 2 vessels tap water with 0,1 mM IAA, 4 contained tap water with 1-methyl-L-histidine on 0,01, 0,001, 0,0001 and 0,00001 mM concentration respectively, 4 contained tap water with 0,1 mM IAA and additionally 1-methyl-L-histidine in the concentrations mentioned. The pH of all solutions was near 6, 7, their temperature was 25° C.

Light was emitted by fluorescent light tubes which gave 10.000 lx at the level of the plants, from 7<sup>h</sup> to 19<sup>h</sup>.

The observations were made on cells in the middle part of the midrib of a detached leaf under a microscope with 400 fold magnification. The experiments were carried out from 10<sup>h</sup>30 to 17<sup>h</sup>30. The observations on leaves from the same concentration of 1-methylhistidine but without and with IAA were usually made successively.

On the first day of an experiment the *Elodea* shoots were put into the solutions. On the 2. and on the 3. day the percentage of cells with systrophe of the chloroplasts and the percentage of cells with no protoplasmic streaming was established in 5 leaves out of every solution. There are some cells with systrophe with streaming and there are some without systrophe and without streaming. On the 4. and 5. day the streaming velocity was measured on 5 cells on every one of 5 leaves out of every solution.

### Results

As can be seen from table 1 there are few systrophes in water, much more in 0.1 mM IAA, in 1-methylhistidine their number decreases with falling concentration and gets to nought in the 2 lowest concentrations. In the combined solutions of 0.1 mM IAA and 0.01 mM 1-methylhistidine there are considerably more systrophes as in either of the single solutions. A plausible explanation will be given in the conclusions. In the combined solution of 0.1 mM IAA and 10<sup>-3</sup> mM 1-methylhistidine there are less systrophes as in either of the single solutions and in the combinations of 0.1 mM IAA with 10<sup>-4</sup> or 10<sup>-5</sup> mM 1-methylhistidine there are no systrophes, what is much less as in 0.1 mM IAA alone. Obviously the systrophe inducing effect of either substance is compensated by the other.

The mean percentage of cells without protoplasmic streaming on the 2. and 3. day of the experiment is listed in table 2. The number of cells without streaming is smallest in water, appreciably higher in 0.1 mM IAA and high in all concentrations of 1-methylhistidine. In the combined solution of 0.1 mM IAA and 0.01 mM 1-methylhistidine there are a little more cells without streaming as in the solution of 0.01 mM 1-methylhistidine alone. A plausible explanation will be given in the conclusions. In the combined solutions of 0.1 mM IAA and 10<sup>-3</sup> to 10<sup>-5</sup> mM 1-methylhistidine the percentual number of cells without streaming is significantly lower as that in 0.1 mM IAA or 1-methylhistidine in respective concentrations alone, as shown in table 2. This suggests that either component compensates the streaming hindering effect of the other.

The streaming velocities on the 4. and 5. day of the experiments are given in table 3. In 1-methylhistidine the streaming velocities rise with falling concentrations of the solutions. In the range of 10<sup>-2</sup> to 10<sup>-4</sup> mM 1-methylhistidine the combination with 0.1 mM IAA augments the streaming velocity appreciably and the velocities are a little higher as in 0.1 mM

Table 1

Percentage of cells showing systrophe of the chloroplasts (mean of counts on 20 leaves from 4 experiments.). Mean values and their differences between various treatments, the standard errors and the probability of error (P). (MH = 1-methylhistidine, IAA = 3-indole-acetic acid)

MH (mM)	%	MH (mM) + 0,1 mM IAA	Difference 1		Difference 2	
			MH - (MH + IAA)	(P)	IAA - (MH + IAA)	(P)
10 <sup>-2</sup>	18 ± 6	10 <sup>-2</sup>	-21 ± 12	(0,07)	-15 ± 13	(0,24)
10 <sup>-3</sup>	15 ± 6	10 <sup>-3</sup>	8 ± 7	(0,22)	17 ± 9	(0,07)
10 <sup>-4</sup>	0	10 <sup>-4</sup>	—		24 ± 9	(0,007)
10 <sup>-5</sup>	0	10 <sup>-5</sup>	—		24 ± 9	(0,007)
H <sub>2</sub> O	2 ± 2	0				

Table 2

Percentage of cells without protoplasmic streaming (mean of counts on 36 leaves from 7 experiments). Mean values and their differences between various treatments, the standard errors and P (symbols see Table 1)

MH (mM)	%	MH (mM) + 0,1 mM IAA	Difference 1		Difference 2	
			MH - (MH + IAA)	(P)	IAA' (MH + IAA)	(P)
10 <sup>-2</sup>	50 ± 6	10 <sup>-2</sup>	-2 ± 9	(0,84)	-18 ± 8	(0,035)
10 <sup>-3</sup>	20 ± 4	10 <sup>-3</sup>	10 ± 5	(0,02)	24 ± 7	(0,0002)
10 <sup>-4</sup>	43 ± 7	10 <sup>-4</sup>	26 ± 8	(0,002)	17 ± 7	(0,009)
10 <sup>-5</sup>	26 ± 6	10 <sup>-5</sup>	15 ± 7	(0,024)	23 ± 5	(0,0002)
H <sub>2</sub> O	8 ± 2	0				

Table 3

Velocities of protoplasmic streaming (mean of 5 experiments, in water 6, in IAA 7 experiments) with their standard errors, the differences between the treatments and P. The value of each experiment is computed from counts of 5 leaves, on every leaf the streaming in 5 cells was measured. Hence every figure in the table is based on 125 measurements

MH (mM)	%	MH (mM) + 0,1 mM IAA	%	Difference 1		Difference 2	
				(IAA + MH) - MH	(P)	(IAA + MH) - IAA	(P)
$10^{-2}$	$7,2 \pm 0,8$	$10^{-2}$	$9,3 \pm 0,3$	$2,1 \pm 0,8$	(0,03)	$0,2 \pm 1,4$	(0,92)
$10^{-3}$	$7,3 \pm 0,7$	$10^{-3}$	$10,4 \pm 0,7$	$3,1 \pm 1,0$	(0,018)	$1,2 \pm 1,4$	(0,34)
$10^{-4}$	$8,0 \pm 0,6$	$10^{-4}$	$9,7 \pm 0,6$	$1,7 \pm 0,8$	(0,08)	$0,6 \pm 1,4$	(0,71)
$10^{-5}$	$8,5 \pm 0,5$	$10^{-5}$	$8,4 \pm 0,6$	$-0,1 \pm 0,8$	(0,87)	$-0,9 \pm 1,4$	(0,62)
H <sub>2</sub> O	$9,6 \pm 0,5$	0	$9,1 \pm 0,9$				

IAA alone. It seems that both substances compensate mutually their streaming retarding effects, the compensating effect 0.1 mM IAA being especially clear.

### Conclusions

Generally, our results suggest that an excess of auxin or of 1-methylhistidine, which is the excitatory substance of the *Hydrocharitaceae*, has unfavourable effects on *Elodea*, whereas combinations of both substances are much less unfavourable, even if the components are in high concentrations. It remains to explain why the combination of 0.1 mM IAA with 0.01 mM 1-methylhistidine is so unfavourable in respect to the number of cells with systrophe or without streaming. By courtesy of Mrs. LENZI we got insight in the results of her experiments on *Elodea* with the same apparatus that we used, but of 2 weeks duration of every experiment. She found with 1-methylhistidine maximal streaming velocities in  $10^{-5}$  and  $10^{-4}$  mM solutions, a deep minimum at  $10^{-3}$  mM solution and again higher values in  $10^{-2}$  to 1 mM solutions. We found in  $10^{-3}$  mM 1-methylhistidine the greatest acceleration of streaming by the addition of IAA. It is a plausible explanation of the results of LENZI, that the maximal values in  $10^{-5}$  and  $10^{-4}$  mM solutions are due to the excitation by 1-methylhistidine, that in  $10^{-3}$  mM solution *Elodea* does not have enough IAA to overcome the unfavourable effects and that 1-methylhistidine in  $10^{-2}$  to 1 mM solutions liberates IAA in the plant, so that both substances together induce high streaming velocities. That 1-methylhistidine in these high concentrations really sets free IAA is shown by the findings of LENZI, that the number of adventitious roots is augmented and their length is reduced in these concentrations.

In agreement with this we have to postulate also in our experiments a liberation of IAA in the plant by 0.01 mM 1-methylhistidine and an additional content of 0.1 mM IAA in the solution may lead to an unfavourably high amount of IAA, rising the number of cells with systrophe and of cells without streaming.

Acknowledgment: We thank Prof. Irmtraud THALER for giving us the opportunity to use the outfit of her Laboratory in the Institute for Plant Physiology at the University of Graz, Austria. We thank also Mrs. Ines LENZI for giving us insight in her yet unpublished work on *Elodea*.

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Zeitschrift/Journal: [Phyton, Annales Rei Botanicae, Horn](#)

Jahr/Year: 1982

Band/Volume: [22\\_1](#)

Autor(en)/Author(s): Umrath Karl, Watanabe Shinobu

Artikel/Article: [The Interaction of the Excitatory Substance with Auxin in Elodea. 143-148](#)