Light and Hormones: Interchangeability in the Induction of Nitrate Reductase

Abstract. *Suitable concentrations of kinetin and gibberellic acid permit induction of nitrate reductase in leaves of tobacco in the dark. Hormone sprays eliminate the need for light induction of the enzyme. The concentration of gibberellic acid required for optimal induction varies according to the concentration of kinetin supplied.*

Nitrate reductase (E.C. 1.6.6.3) is an enzyme that is induced by its substrate (*1*). However, the induction of the enzyme requires not only nitrate but also light (*2*) and CO_2 (*3*). It has been suggested that the requirement for light was due to the dependence of the reducing system on reduced nicotinamide-adenine dinucleotide (NADPH). The CO_2 requirement for induction of nitrate reductase was believed to be due to dependence on a photosynthetic product for the synthesis of the enzyme.

The activity of nitrate reductase in tobacco leaves is largely determined by kinetin and gibberellic acid (GA_3) (*4*). Light is not only a source of energy in photosynthesis but seems to relate to the synthesis of gibberellin (*5*). Consequently, we considered that the need for light induction of nitrate reductase may be due to its effect on the concentrations of endogenous growth substances rather than on the production of a photosynthetic cofactor.

Nicotiana rustica (L) plants, germinated in washed vermiculite and thereafter grown in Hoagland solution (½ concentration), were used in our experiments. Young, expanding leaves from 2-month-old plants with leaf-blades 6 to 8 cm long were selected for nitrate reductase determination (*6*). Three such leaves, each taken from three different plants, were treated and extracted together. Growth substances were sprayed on the plants in 0.01 percent Tween solution. Enzyme induction was studied in plants grown for about 2 months in the Hoagland solution and transferred thereafter for 5 days to 0.5 m*M* $CaSO_4$ to obtain plants depleted of nitrate. After depletion of the nitrate, plants were returned to Hoagland solution and placed for 24 hours in a glass house with natural light or in a darkroom maintained at 15°C. Hormones were sprayed on immediately after transferring the plants from $CaSO_4$ to Hoagland solution and enzyme determination was made 24 hours later.

Plants returned to Hoagland solution after 5 days in 0.5 m*M* $CaSO_4$ showed rapid induction of nitrate reductase (0.42 micromole of NO_2^- formed per gram of fresh weight per hour) when kept in a glass house for 24 hours. The activity of the enzyme was much smaller (0.16 micromole of NO_2^- formed per gram of fresh weight per hour) for plants transferred to Hoagland and then kept in darkness for a day. These results support observations (*2*, *3*) on the need for light to induce nitrate reductase.

We then sprayed nitrate-depleted plants with solutions of kinetin and GA_3. The hormones were sprayed on at different concentrations over the entire plant shoots after they had been transferred to Hoagland solution and placed in the darkroom (Table 1). Suitable exogenous additions of kinetin (10 ppm) and GA_3 (200 ppm) (*7*) can replace light entirely.

The hormone combination required for maximum enzyme induction varies from experiment to experiment. This is understandable if one considers that the endogenous concentrations of cytokinin and gibberellin were not identical for each batch of plants used; the endogenous concentrations of the hormones are established by environmental conditions which were very variable in the glass house in which plants were grown. Although we show in Table 1 that 95 percent of the induction taking place in light is obtained by application of the hormones in the dark, we have observed instances of up to 133 percent. This shows that the effect of light is not optimal and that induction may be increased by the addition of adequate amounts of hormones. This assumption has been demonstrated when growth substances have been sprayed on tobacco plants growing in light (*4*). The variations of response observed are due to the fact that plants were grown in a naturally varying environment and not in a controlled environment. Endogenous concentrations of hormones presumably change from experiment to experiment and so does the extent of response to a given application of growth substances within the limits previously indicated (95 to 133 percent of control). There seems to be a delicate interaction between both substances on the synthesis of the enzyme, the optimum concentration of GA_3 varying with the concentration of kinetin supplies. In all the experiments we observed that application of low concentrations of GA_3 inhibits the induction of nitrate reductase, especially when exogenous kinetin was not added or when it was supplied at low concentrations. This phenomenon should be taken into consideration to understand the possible nature of the interaction between cytokinins and gibberellins.

Table 1. Effect of kinetin and gibberellic acid (GA_3) on the induction of nitrate reductase (micromoles of NO_2^- formed per gram of fresh weight per hour) in darkness. Hormones were sprayed over plants before transfer to Hoagland solution. Enzyme determinations were made after 24 hours.

Additions (ppm)		Enzyme activity	Percent of control
Kinetin	GA_3		
		Light	
None		0.97	100
		Darkness	
None		0.24	25
1		0.50	52
1	100	0.04	4
1	200	0.26	27
1	400	0.52	54
1	800	0.44	45
10		0.25	26
10	100	0.60	62
10	200	0.92	95
10	400	0.40	41
10	800	0.01	1

Although the results (Table 1) indicate that kinetin and GA_3 affect the induction of nitrate reductase, one cannot discard possible interactions with other growth substances which may affect the concentration of the enzyme in vivo.

The need for CO_2 for induction of nitrate reductase observed in Perilla leaves (*3*) seems to be indirect. Kannagara and Woolhouse (*3*) pointed out that sugar does not replace the need for light plus CO_2 in nitrate reductase induction. Ben-Zioni *et al.* (*8*) showed that the reduction of nitrate is linked to a concomitant synthesis of malate. Carbon dioxide allows the reduction of nitrate in vivo through its participation in the synthesis of malic acid, which is necessary to avoid extreme *p*H changes in the tissue. The functional coupling of these two reactions is necessary in vivo to replace the inorganic anion being reduced (NO_3^-) by the production of an organic substitute (malate). If the synthesis of malate is arrested by lack of CO_2, reduction of nitrate will probably stop.

We suggest, therefore, that light is

necessary for nitrate reductase induction (or synthesis) in leaves of tobacco because of its effect on the concentrations of one or more endogenous growth regulators. This effect may be due to either stimulation of synthesis or retardation of breakdown of those hormones required for nitrate reductase synthesis.

S. HERMAN LIPS
N. ROTH-BEJERANO
Negev Institute of Arid Zone Research, Department of Plant Physiology, Beer-Sheva, Israel

References and Notes

1. M. I. Candella, E. G. Fisher, E. J. Hewitt, *Plant Physiol.* **32**, 280 (1957).
2. R. H. Hageman and D. Flesher, *ibid.* **35**, 700 (1960).
3. C. G. Kannagara and H. W. Woolhouse, *New Phytol.* **66**, 553 (1967).
4. N. Roth-Bejerano and S. H. Lips, *ibid.*, in press.
5. D. Köhler, *Planta* **69**, 27 (1966).
6. A. Bar-Akiva and J. Sagiv, *Physiol. Plant.* **20**, 500 (1967).
7. Kinetin (6 furfurylamino purine) was obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Gibberellic acid (GA_3) was supplied by Riedel de Haen A.G., Seelze-Hanover, Germany.
8. A. Ben-Zioni, S. H. Lips, Y. Vaadia, in preparation.

3 June 1969; revised 14 July 1969

Visceral and Behavioral Responses to Intraduodenal Fat

Abstract. *Introduction of milk or corn oil into the duodenum of the cat evokes an increase in superior mesenteric blood flow (blocked by atropine), an inhibition of gastric and duodenal motility, and sedation. Cholecystokinin-pancreozymin mimics the mesenteric vascular effect of intraduodenal fat and seems to have a sedating action.*

Increased blood flow in the mesenteric artery occurs in dogs after a meal (*1*). We now report that in cats this increased flow depends in part on stimuli arising in the duodenum, and that these stimuli elicit other characteristic autonomic and behavioral responses.

Under sterile conditions, the following devices were implanted in eight cats in a single-stage operation: (i) noncannulating electromagnetic flowmeter probes (Micron) around the superior mesenteric and external iliac arteries for recording of blood flow via Biotronex electromagnetic flowmeters, along with miniature hydraulic occluders for transient occlusion of the vessels to obtain zero-flow levels; (ii) polyvinyl cannulas in the inferior mesenteric artery and jugular vein for pressure recordings via Statham pressure transducers; (iii) polyvinyl cannulas inserted through the wall of the stomach and ending in the gastric antrum and at three levels in the duodenum, for recording motility (pressure) changes and for instillation of various substances; (iv) skull and deep, stereotaxically oriented, electrodes for electroencephalograms (EEG); and (v) neck and periorbital intramuscular electrodes for electromyograms. The cannulas and flowmeter cables were contained inside a small pack at the interscapular region, after having been tunneled along the skin of the back from the lumbar region. The head electrodes were connected to a miniature socket anchored to the skull with stainless steel wire and dental cement. Recordings were made on a Grass polygraph model VII and a Grass electroencephalograph model IV, with common time and signal marking.

At each recording session (9:30 a.m. to 5:00 p.m.) an animal was placed in a ventilated, lighted box, 40 by 60 by 80 cm, with a one-way window. Food and water were not available to the animal during this period. Generally, the animals had free access to food and water in their cages until placed in the recording box. Observations began 7 to 10 days after the surgery and could be continued for 1 to 4 months.

Introduction of milk (5 ml) into the duodenum produced (i) a 50 to 100 percent increase in superior mesenteric blood flow, starting in 3 to 6 minutes and lasting for 30 to 60 minutes, which was unaccompanied by any changes in heart rate, arterial pressure, or iliac

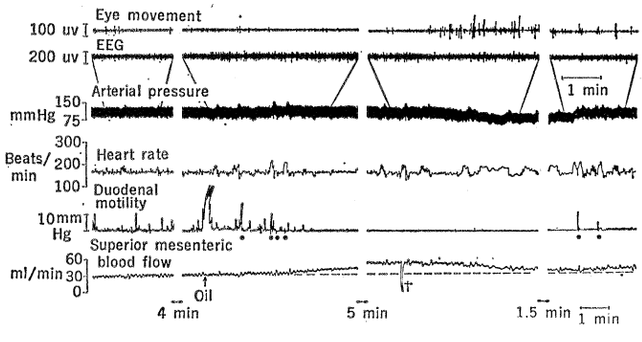

Fig. 1 (left). Response to injection of 1.0 ml of corn oil into the second part of the duodenum in an awake male cat. Diagonal lines connecting EEG and arterial pressure tracings indicate points of simultaneity between events recorded on electroencephalograph (eye movement and EEG) and polygraph (other parameters). Heart rate was recorded via Grass tachograph from pressure pulses. Dots beneath duodenal motility record indicate movement artifacts. Cross beneath lowest tracing indicates zero blood flow during arterial occlusion. Dashed line is drawn for convenience to indicate control level of blood flow. Time intervals between the selected segments of the continuous record are indicated at the bottom.

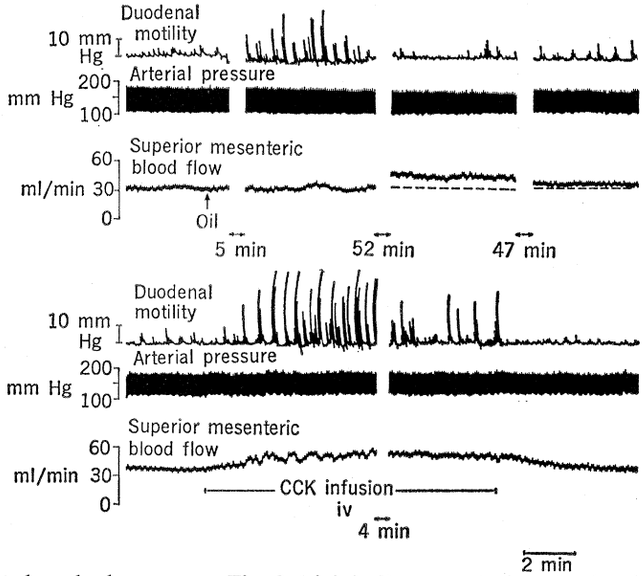

Fig. 2 (right). Response to intraduodenal injection of 1.5 ml of corn oil in a chloralose-anesthetized male cat (4.1 kg) (top). Response in the same cat to intravenous infusion of CCK-PZ (4.7 unit kg^{-1} $hour^{-1}$) (bottom). Dashed line and time interval notations as in Fig. 1.