The nodal membrane of amphibian and mammalian myelinated nerve fibers contains sodium channels with a high surface density of 1000–2000/μm^2. The corresponding average distance 20–30 nm between channels is so small that interactions between neighboring channels could occur. Indeed, the conductance of a single sodium channel in a frog nerve fiber is higher when the number of activatable channels is reduced either by a more positive holding potential or by the addition of the sodium-channel blocker tetrodotoxin[7]. This seems to indicate that the ion flux through one open channel is hindered by the ion flux through neighboring channels. The nodal membrane is an ideal preparation to study such channel-channel interactions because of the extremely high channel density. Yet the total channel number per node is still low enough to allow the simultaneous measurement of sodium currents and sodium-current fluctuations with conventional analog-to-digital converters. Interactions between neighboring ionic channels could affect not only their conductances but also the gating kinetics of individual channels. It would be of great interest to investigate whether peculiarities of the kinetics of ionic currents in myelinated nerve (e.g. the pronounced non-exponential sodium inactivation) could originate in such interactions. It also seems to be promising to study possible channel-channel interactions in other membranes with a lower overall channel surface density. Significant interactions would then indicate that the channels are not equally distributed over the membrane but are probably arranged within local clusters of more channels.

The above list of still unexplained properties of myelinated nerve fibers is by no means complete, and the selection of the topics has been biassed by the author's own electrophysiological investigations. It is hoped, however, that the examples mentioned in this chapter and the contents of all previous sections of this multi-author review have illustrated the close interrelations between the electrical and structural aspects of myelinated nerve fibers and the importance of future electrophysiological and morphological investigations which may contribute to a better understanding of the function of myelinated nerve fibers.

1 Chiu, S.Y., Inactivation of sodium channels: Second order kinetics in myelinated nerve. J. Physiol. *273* (1977) 573–596.
2 Chiu, S.Y., Ritchie, J.M., Rogart, R.B., and Stagg, D., A quantitative description of membrane currents in rabbit myelinated nerve. J. Physiol. *292* (1979) 149–166.
3 Conti, F., Neumcke, B., Nonner, W., and Stämpfli, R., Conductance fluctuations from the inactivation process of sodium channels in myelinated nerve fibres. J. Physiol. *308* (1980) 217–239.
4 Dubois, J.M., Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane. J. Physiol. *318* (1981) 297–316.
5 Huxley, A.F., and Stämpfli, R., Direct determination of membrane resting potential and action potential in single myelinated nerve fibres. J. Physiol. *112* (1951) 476–495.
6 Neumcke, B., and Stämpfli, R., Sodium currents and sodium-current fluctuations in rat myelinated nerve fibres. J. Physiol. *329* (1982) 163–184.
7 Neumcke, B., and Stämpfli, R., Alteration of the conductance of Na^+ channels in the nodal membrane of frog nerve by holding potential and tetrodotoxin. Biochim. biophys. Acta *727* (1983) 177–184.
8 Schwarz, J.R., Bromm, B., and Ochs, G., Inactivation of sodium permeability in motor and sensory nerve fibres. Pflügers Arch. *389* (1981) R 23.
9 Sigworth, F.J., The variance of sodium current fluctuations at the node of Ranvier. J. Physiol. *307* (1980) 97–129.
10 Stämpfli, R., Is the resting potential of Ranvier nodes a potassium potential? Ann. N.Y. Acad. Sci. *81* (1959) 265–284.

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Full Papers

The effect of digitoxose on feeding behavior

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Summary. The effect of digitoxose (DIG) on food intake, gold thioglucose (GTG) lesion formation in the ventromedial hypothalamus (VMH), and VMH glucose oxidation in vitro was investigated in mice. DIG significantly decreased the amount of food ingested during the day compared to controls ($p < 0.01$). DIG had no effect on nocturnal feeding. GTG lesion formation in the VMH and VMH glucose oxidation were not altered by DIG treatment. These results suggest that DIG alters daytime feeding behavior by affecting extrahypothalamic or peripheral glucoreceptor sites.

Mayer[22] proposed the original glucostat hypothesis on the basis of gold thioglucose (GTG) vulnerability of the ventromedial hypothalamus (VMH) coupled with subsequent hyperphagia. These data suggested that a mechanism for regulating food intake, which was sensitive to glucose analogs, was located in the VMH. GTG sensitivity in the VMH and subsequent lesion formation can be inhibited by systemic injections of 2-deoxy-D-glucose (2DG)[19]. 2DG is a competitive inhibitor of glucose utilization[29,30], which also induces hyperphagia[19]. Phlorizin, a competitive inhibitor of glucose transport[20], also will block GTG necrosis in the VMH[4,9] and induce hyperphagia[13]. Pharmacologic manipulation of VMH glucose utilization may affect feeding behavior. Smith and Epstein[28] have proposed a glucoprivic mechanism for the control of feeding behavior; brain glucoreceptors stimulate feeding when their glucose utilization is diminished. Since Marrazzi[21] has demonstrated the presence of glucose-sensitive neurons in the VMH, a glucoreceptor system may exist in the VMH which is important in controlling food intake.

However, specific GTG vulnerability in the VMH has not been widely accepted. Liebelt and Perry[18] presented evidence that the GTG-induced VMH lesion is nonspecific. They noted that other hypothalamic and extrahypothalamic nuclei can be damaged by GTG, and attributed this general destruction to blood-brain barrier damage. Caffyn[5,6] examined GTG-induced lesions by electron microscopy and concluded that GTG caused capillary damage, resulting in increased capillary permeability and subsequent downstream ischemia. These conditions produced edema and cellular damage. Levine and Sowinski[17] found that the polyamine, 3,3′-methylimino-bis-(N-methylpropylamine), produced VMH lesions very similar to those of GTG, even though the compounds are structurally unrelated. This evidence suggested that GTG is a drug which caused nonspecific lesions of the VMH via vascular damage.

Recently, Young et al.[31] have observed that, while regions of the hypothalamus other than the VMH may be affected by GTG, the median eminence is not damaged. They concluded that GTG may have a specificity for certain hypothalamic structures and suggested GTG lesions might result from impairment of glucose uptake and utilization by the VMH. Debons et al.[10] have demonstrated that damage to the neuropil in the pericapillary regions occurs prior to observable capillary damage and is restricted to a small area of the VMH. They have suggested that GTG is specific, damaging target neuronal structures in the VMH before ischemia and edema develop.

Recent studies of VMH glucose oxidation in vitro have revealed that several glucose analogs (unpublished observations), including 2DG, and inhibitors of glucose transport, such as phlorizin[4] do not inhibit glucose oxidation significantly except at very high concentrations. This result is consistent with the observations of Likuski et al.[19] that large doses of 2DG are required to block GTG lesion formation in the VMH in vivo. Brown and Viles[4] have shown that large systemic doses of phlorizin also were required to block GTG lesion formation in the VMH in vivo. However, the high concentrations of these inhibitors necessary to affect VMH glucose oxidation in vitro suggest that these compounds are not very effective inhibitors of glucose oxidation, and possibly not highly competitive glucoreceptor-binding analogs, at least in the VMH.

The sugar digitoxose, 2,3-dideoxy-ribohexose, part of the cardiac glycoside digitoxin, has been shown to inhibit glucose-stimulated insulin release from the pancreatic islets without affecting glucose oxidation in the beta cells. Garcia Hermida and Gomez-Acebo[12] attributed this phenomenon to the intereaction of digitoxose (DIG) with a glucoreceptor in the beta cell membrane, which did not interfere with glucose transport or oxidation. This proposal suggested 2 pathways for glucose utilization in this system: one affecting stimulus-secretion coupling and the other providing glucose for energy utilization.

In this study, DIG was used as a chemical probe to test several aspects of VMH glucose sensitivity for evidence of differentiated glucose utilization, as proposed for the pancreas. The experiments included: 1. measuring the effects of DIG on feeding behavior as an indicator of modulation of feeding behavior, 2. the effects of DIG on GTG-induced necrosis as an indication of inhibition of GTG binding in the VMH, and 3. the effects of DIG on VMH glucose oxidation as an indicator of inhibition of glucose utilization.

Materials and methods

CF1 female mice (Charles River Breeding Labs), weighing 20–25 g, were used for these experiments. The mice were fed Tek-Lab Mouse and Rat Diet and given tap water freely. A photoperiod of 12 h light: 12 h dark (lights on 08.00 h), and an environmental temperature of 23 °C were maintained. A 1000 mg/kg DIG dose was given. If more than 1 injection was used, the first 4 injections were given at 3-h intervals. Subsequent administrations were given every 6 h. GTG was given in a dose of 300 mg/kg. All injections were given i.p. The Mann-Whitney U and t test were used to test for significant differences.

Feeding behavior

Mice were assigned randomly to 2 groups: normal and obese. Within each group were controls (water injections, $n=4$) and experimentals (DIG injections, $n=4$). Since food intake can vary tremendously between the light and dark periods, the effects of DIG were assessed under both light and dark conditions. Under dark conditions, all injections and weighings

were done with red light. To enhance feeding, the animals were deprived of food for 18 h prior to experimentation[16]. The light and dark experiments were begun 2 h after the end of the previous 12-h photoperiod. The control (water) and DIG injections were given 15 min before food (Tek-Lab Mouse and Rat Diet) was made available, and at 10.00 h for day experiments and 22.00 h for night experiments. Obese mice were derived from normal mice given a GTG injection. These GTG-treated mice were judged obese if they weighed 25% or more than their uninjected littermates 6 weeks postinjection. Total food intake and group body weight were monitored every hour for the 6-h period. To determine whether or not the animal colony was responsive to drug-induced alterations of food intake, mice were selected at random from the stock colony, injected with 2DG (300 mg/kg) at approximately 10.00 h, and monitored for hyperphagia. All of these 2DG-treated mice (n = 8) became hyperphagic. The mice were thus considered responsive to drug-induced changes in feeding behavior.

DIG and GTG interaction

DIG was injected 30 min before the GTG injection, between 10.00 h and 12.00 h. After the GTG injection, the mice (n = 10) were subsequently injected with DIG at the appropriate intervals. A total of 6 DIG injections were given. Control animals (n = 10) were given H_2O instead of DIG. The mice were decapitated 24 h after the GTG challenge. The brains were removed from the skull, fixed in Bouin's fluid, dehydrated, and embedded in paraffin. 7-µm-thick sections were made through the VMH at the level of the median eminence and stained with hematoxylin and eosin. The VMH was examined for lesions by light microscopy.

In vitro glucose oxidation studies

Animals were decapitated in the morning and the VMH was dissected free from the brain at the level of the median eminence as a cube of tissue. Two VMH tissue cubes (caps) were placed in a culture vessel (a 25-ml Erlenmeyer flask with a gas trap; n = 6) containing 1.47 ml of medium, 7.75 mg DIG (Sigma) in H_2O, and 7.5 µCi of aqueous uniformly labeled [^{14}C] D-glucose (Schwartz-Mann). Control vessels (n = 6) contained saline instead of DIG. The incubating media was similar to cerebral spinal fluid and prepared according to Jones et al.[12]. Other vessels possessed no tissue and served as controls (n = 6) for auto-oxidation of the [^{14}C] D-glucose. The caps were allowed to incubate with DIG in the medium 15 min at 37 °C. The reaction was stopped and the [^{14}C] CO_2 released by the addition of 9 N H_2SO_4 (0.2 ml) to the medium. Hyamine (Sigma) (0.5 ml) was added to the gas trap to absorb the [^{14}C] CO_2 and incubated for an additional 60 min. The hyamine was collected from each vessel and [^{14}C] emissions counted with a Beckman 250 liquid scintillation counter. As a test for tissue viability, saline vessels with hypothalamic tissue were supplemented with 15 µl of 2 mM dinitrophenol (Fisher) in an aqueous solution and processed as described above.

Results

Feeding behavior

The effect of DIG on short-term feeding behavior (a 6-h interval) was examined under light and dark conditions. DIG was not effective in significantly altering food intake in the dark. However, in the light DIG reduced short-term food intake significantly, $p < 0.01$ (table). The effect of DIG on short-term feeding was also tested in mice made obese with GTG. DIG had no effect on short-term feeding in GTG-treated obese mice during the light. Thus, the reduced food intake observed in normal mice in the light was abolished by a GTG challenge.

Normal mice responded typically to changes in the photoperiod; more food was consumed at night than during the day ($p < 0.01$). Also, the obese mice ate more food than the normals ($p < 0.01$).

DIG inhibition of GTG necrosis

DIG injections did not alter the vulnerability of the VMH to GTG administration. Mice injected with either DIG and GTG or water and GTG exhibit GTG lesions of similar dimensions (fig. 1). No significant difference existed in lesion size between the 2 treatments ($p > 0.05$), as determined by planimetry.

In addition, preliminary data indicated that DIG infused into the VMH did not interfere with GTG necrosis. These intrahypothalamic infusions of DIG did not eliminate or alter size of the GTG lesion.

Effect of DIG on food intake in food-deprived mice in light or dark

	Normal mice food intake (g/g body weight, mean ± SE)		GTG obese mice food intake (g/g body weight, mean ± SE)	
	Water (control, n = 4)	DIG (n = 4)	Water (control, n = 4)	DIG (n = 4)
Light	0.088 ± 0.005 *	0.073 ± 0.007	0.132 ± 0.048	0.134 ± 0.050
(Range)	(0.085–0.092) *	(0.064–0.077) *	(0.080–0.176)	(0.088–0.188)
Dark	0.108 ± 0.012	0.111 ± 0.012		
(Range)	(0.096–0.119)	(0.103–0.125)		

* A significant difference between treatments, $p < 0.01$.

In vitro glucose oxidation

DIG enhanced glucose oxidation in the VMH by almost 13% at concentrations 3 times greater than glucose in the incubation medium (fig. 2). However, this difference was not significant ($p > 0.5$). The effect of dinitrophenol, an uncoupler of oxidative phosphorylation, was similar to that observed earlier[3,4]. Glucose oxidation increased approximately threefold, indicating that glucose oxidation pathways and regulatory mechanisms were still functional.

Discussion

In this study, it has been demonstrated that the sugar DIG has no effect on GTG-induced lesion formation in the VMH, and in vitro glucose oxidation in VMH tissue. However, DIG injections did decrease daytime food intake significantly without affecting nocturnal feeding behavior.

Debons et al.[7,8,11] have shown that VMH vulnerability to GTG-induced necrosis is insulin-dependent; diabetic mice are resistant to GTG lesion formation. DIG has been shown to inhibit glucose-stimulated insulin release from the pancreas[12]. Thus, if DIG decreased insulin levels sufficiently, GTG lesion formation should be inhibited in DIG-treated mice. However, DIG injections did not prevent the development of a GTG-induced lesion or alter the size of the lesion, nor did an intrahypothalamic infusion of DIG. The possibilities of DIG binding competitively to GTG-sensitive neural tissue or diminishing insulin secretion, and thereby inhibiting GTG lesion formation, is not supported.

VMH caps incubated in vitro with DIG showed no inhibition of glucose oxidation; instead they showed an enhanced (albeit insignificant glucose oxidation rate, about 13% greater than the controls. Garcia Hermida and Gomez-Acebo[12] observed a similar enhancement of glucose oxidation in pancreatic islets treated with DIG. This evidence suggested that DIG is not an effective inhibitor of glucose oxidation in the VMH, as has been observed with a number of glucose analogs (unpublished observations). Indeed, phlorizin, an inhibitor of glucose transport, showed a significant potentiation of VMH glucose oxidation in vitro, but only at a low concentration[4].

DIG significantly reduced food intake in mice during the light photoperiod, but had no effect on feeding in the dark. It might be expected that an effect would be more noticeable with darkness, since mice consume the majority of their food at night. However, this photoperiod-dependent difference in feeding behavior could be explained by a hypothalamic circadian rhythm. The suprachiasmatic nucleus in the hypothalamus, an area associated with the control of diurnal variations, selectively bound 2DG during the day but not at night[27]. In addition, Larue-Achagiotis and LeMagnen[15] have shown that a 2DG challenge inhibited food intake at night but stimulated feeding

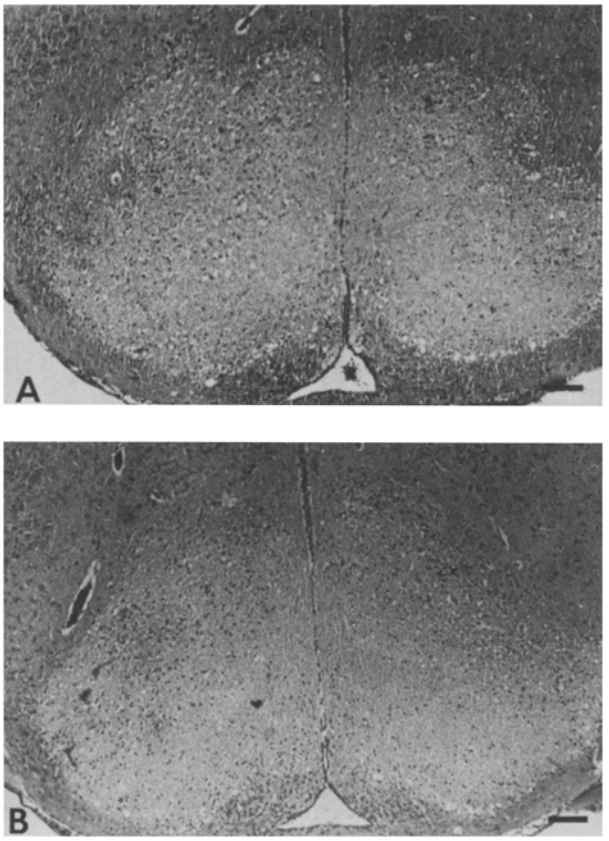

Figure 1. Cross sections of mouse brain, depicting the VMH at the level of the median eminence, 24 h after a GTG challenge. *A* Water given i.p. initially followed by GTG (300 mg/kg) i.p. *B* DIG (1000 mg/kg) injected i.p. followed by 300 mg/kg GTG parenterally. Bar equals 1.0 mm.

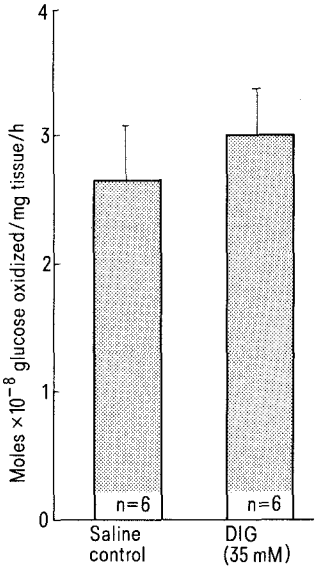

Figure 2. The effect of DIG on glucose oxidation in the VMH in vitro. The bars indicate the mean±SE. No significant difference is apparent.

during the day. The daytime results from their study[15] are incongruent with our data. This inconsistency may be linked to the ability of 2DG to be a glucoprivic stimulus, while DIG is not. Ritter et al.[25] have demonstrated that the glucoreceptors responsible for eliciting hyperphagia, under conditions of glucoprivation, are located in the hindbrain and not in the hypothalamus. A glucoprivic stimulus resulting from the administration of 2DG or 5-thioglucose (5TG) is known to produce an increase in food intake[25]. These 2 compounds also inhibit GTG lesion formation, presumably by binding to VMH glucoreceptors (reference for 2DG-19; 5TG-personal observations). Since DIG has an opposite effect on feeding behavior compared with these glucoprivic compounds and DIG has no effect on GTG-induced necrosis in the VMH, apparently DIG cannot be described as a substance capable of inducing glucoprivation or binding to hypothalamic glucoreceptors. DIG probably does not affect daytime feeding behavior by a central mechanism.

Garcia Hermida and Gomez-Acebo[12] demonstrated that DIG affected glucose-stimulated insulin secretion, presumably by binding to beta cell membrane glucoreceptors. It has been well-documented that peripheral hepatic glucoreceptors play a role in the regulation of feeding behavior[24,26]. The exact mechanism is unclear, but these peripheral glucoreceptors may produce feeding and/or satiety signals, which are relayed to the brain via vagal afferents[23]. Phlorizin, when infused intraventricularly into the brain, produced hyperphagia, apparently by binding to central glucoreceptors[13]. An infusion of phlorizin into the liver decreased the vagal afferent discharge rate, probably by affecting hepatic glucoreceptors[23]. Furthermore, infusions of 2DG into the hepatic portal system stimulated feeding[24,26]. Since DIG is known to affect insulin secretion via glucoreceptors, it is conceivable that DIG may affect other peripheral glucoreceptors, such as those found in the liver. Thus, our observation that DIG inhibited daytime feeding behavior may be due to a diurnal alteration of vagal afferent discharge mediated by hepatic glucoreceptors. Further investigations are needed to explore this possibility.

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3 Brown, D.F., and Viles, J.M., Glucose oxidation in the ventromedial hypothalamus is not affected by insulin or ouabain but depressed by alloxan treatment. Exp. Brain Res. *40* (1980) 464-467.

4 Brown, D.F., and Viles, J.M., Systemic phlorizin prevents gold thioglucose necrosis in the ventromedial hypothalamus. Brain Res. Bull. *8* (1982) 347-351.

5 Caffyn, Z.E.Y., Ultrastructural changes induced in the hypothalamus of the mouse by gold thioglucose. Br. J. exp. Path. *52* (1971) 517-523.

6 Caffyn, Z.E.Y., Early vascular changes in the brain of the mouse after injections of gold thioglucose and bipiperidyl mustard. J. Path. *106* (1972) 49-57.

7 Debons, A.F., Krimsky, I., and From, A., A direct action of insulin on the hypothalamic satiety center. Am. J. Physiol. *219* (1970) 938-943.

8 Debons, A.F., Krimsky, I., From, A., and Cloutier, R.J., Rapid effects of insulin on the hypothalamic satiety center. Am. J. Physiol. *217* (1969) 1114-1118.

9 Debons, A.F., Krimsky, I., From, A., and Pattinian, H., Phlorizin inhibition of hypothalamic necrosis induced by gold thioglucose. Am. J. Physiol. *226* (1974) 574-578.

10 Debons, A.F., Krimsky, I., From, A., Siclari, E., Maayan, M.L., Fani, K., and Jimenez, F.A., Action of gold thioglucose on pericapillary structures in the ventromedial hypothalamus. J. Path. *129* (1979) 73-81.

11 Debons, A.F., Krimsky, I., Likuski, H.J., From, A., and Cloutier, R.J., Gold thioglucose damage to the satiety center: inhibition in diabetes. Am. J. Physiol. *214* (1968) 652-658.

12 Garcia Hermida, O., and Gomez-Acebo, J., Digitoxose and the existence of a glucoreceptor in the beta cells of the islets of langerhans of the rat. Biochem. biophys. Res. Commun. *62* (1975) 524-530.

13 Glick, Z., and Mayer, J., Hyperphagia caused by cerebral ventricular infusions of phlorizin. Nature *219* (1968) 1374.

14 Jones, M.T., Hillhouse, E., and Burden, J., Secretion of corticotropin-releasing hormone in vitro, in: Frontiers in Neuroendocrinology, vol.4, pp.195-226. Eds L. Martini and W.F. Ganong. Raven Press, New York 1975.

15 Larue-Achagiotis, C., and LeMagnan, J., Dual effects of 2-deoxy-D-glucose on food intake in the rat: inhibition at night and stimulation in the daytime. Physiol. Behav. *23* (1979) 865-870.

16 Latham, C.J., and Blundell, J.E., Evidence for the effect of tryptophan on the pattern of food consumption in free feeding and food deprived rats. Life Sci. *24* (1979) 1971-1978.

17 Levine, S., and Sowinski, R., Hypothalamic and medullary lesions caused by an aliphatic triamine unrelated to gold thioglucose. J. Neuropath. exp. Neurol. *41* (1982) 54-66.

18 Liebelt, R.A., and Perry, J.H., Action of gold thioglucose on the central nervous system, in: Handbook of Physiology Alimentary Canal, vol.1, pp.271-285. American Physiological Society, Washington DC 1967.

19 Likuski, H.J., Debons, A.F., and Cloutier, R.J., Inhibition of gold thioglucose-induced hypothalamic obesity by glucose analogues. Am. J. Physiol. *212* (1967) 669-676.

20 Lotspeich, W.D., Phlorizin and the cellular transport of glucose. Harvey Lect. *56* (1961) 63-91.

21 Marrazzi, M.A., Hypothalamic glucoreceptor response: biphasic nature of unit potential changes, in: Hunger: Basic Mechanisms and Clinical Implications, pp.171-178. Eds D.W. Novin, W. Wyrwicka, and G. Bray. Raven Press, New York 1976.

22 Mayer, J., Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. Ann. N.Y. Acad. Sci. *63* (1955) 15-43.

23 Niijima, A., Afferent impulse discharges from glucoreceptors in the liver of the guinea-pig. Ann. N.Y. Acad. Sci. *157* (1969) 690-700.

24 Novin, D., VanderWeele, D.A., and Rezek, M., Infusions of 2-deoxy-D-glucose into the hepatic portal system causes eating: evidence for peripheral glucoreceptors. Science *181* (1973) 858-860.

25 Ritter, R.C., Slusser, P.G., and Stone, S., Glucoreceptor controlling feeding and blood glucose: location in the hindbrain. Science *213* (1981) 415-453.

26 Russek, M., Demonstration of the influence of an hepatic glucosensitive mechanism on food intake. Physiol. Behav. *5* (1970) 1207-1209.

27 Schwartz, W.J., and Gainer, H., Suprachiasmatic nucleus: use of ^{14}C-labelled deoxyglucose uptake as a functional marker. Science *197* (1977) 1089–1091.
28 Smith, G.P., and Epstein, A.N., Increased feeding in response to decreased glucose utilization in the rat and monkey. Am. J. Physiol. *217* (1969) 1083–1087.
29 Wicks, A.N., Drury, D.R., and Mortia, T.N., 2-Deoxyglucose, a metabolic block for glucose. Proc. Soc. exp. Med. *89* (1955) 579–582.
30 Wicks, A.N., Drury, D.R., Nakada, H.I., and Wolfe, J.B., Localization of the primary metabolic block produced by 2-deoxyglucose, J. biol. Chem. *224* (1957) 963–969.
31 Young, J.K., Nance, K.M., and Gorski, R.A., Effects of diet upon hypothalamic vulnerability of gold thioglucose in male mice. Brain Res. Bull. *4* (1979) 69–74.

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In vitro analysis of prothoracicotropic hormone specificity and prothoracic gland sensitivity in Lepidoptera[1]

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Summary. The kinetics of prothoracicotropic hormone (PTTH) activation of pupal prothoracic glands (PG) of the cabbage army worm, *Mamestra brassicae*, and silkworm, *Bombyx mori*, were determined in vitro. Activation was assessed by comparing the increase in the rate of ecdysone synthesis by one member of a PG pair incubated with a PTTH preparation from pupal brains with the basal rate of synthesis of the other PG incubated without PTTH. A time course of ecdysone synthesis revealed that *Bombyx* PTTH extract activated *Bombyx* PG, and *Mamestra* PTTH extract activated *Mamestra* PG. Dose responses of activation of *Bombyx* and *Mamestra* PG by their respective PTTH were saturable and were indicative of neurohormonal activation. The *Bombyx* PG were half-maximally activated (A_{50}) by far less PTTH than *Mamestra* PG, 0.34 and 0.91 brain equivalents, respectively. Heterologous dose response of activation studies, in which PTTH and PG from *Mamestra*, *Bombyx* and the tobacco hornworm, *Manduca sexta*, were assayed for interspecific PG sensitivity and PTTH specificity, revealed cross-reactivity among the three PTTH-PG axes, with *Manduca* PG being more sensitive to the PTTH of the other species and *Bombyx* PTTH being the most effective in activating the PG of the other two species.

The prothoracicotropic hormone (PTTH) is a peptide neurohormone[9,15] synthesized by specific neurosecretory cells in the insect brain[5,15] and released from its neurohemal organ, the corpus allatum[4] in *Manduca sexta*, into the hemolymph at specific times during insect development[9]. Once released, PTTH activates the prothoracic glands (PG) to synthesize and release ecdysone which is converted to 20-hydroxyecdysone by tissues other than the PG[16,19]. It is 20-hydroxyecdysone, or possibly a combination of ecdysone and 20-hydroxyecdysone, that elicits the molting process. Juvenile hormone acts in conjunction with the ecdysteroids as a modulator of cellular events in target tissues[16].

The ecdysteroids and juvenile hormones from a variety of insects have been characterized and these hormones appear to be ubiquitous, exhibiting only subtle molecular heterogeneity[16]. Although the chemical characterization of PTTH has been a matter of intensive study, the structure of this elusive peptide has not yet been elucidated[8,9,15]. Therefore, the only available information pertaining to the degree of structural conservation of PTTH among insect species comes from classical brain transplantation studies and in vivo bioassays, which suggest that this neurohormone may be far less conserved structurally than are the ecdysteroids and juvenile hormones[14].

With the development of a sensitive and specific in vitro assay for PTTH that quantifies activation of the PG by the neurohormone, it is now possible to investigate the cross-reactivity of PTTH among different insect species without purifying the PTTH of each species. This study was conducted to compare PTTH specificity, as well as PG sensitivity to PTTH, among *Bombyx mori*, *Mamestra brassicae* and *Manduca sexta*, representatives of 3 superfamilies of Lepidoptera; Bombycoidea, Noctuoidea and Sphingoidea.

Materials and methods

Animals. Larvae of the cabbage army worm, *Mamestra brassicae* (Noctuidae), and the tobacco hornworm, *Manduca sexta* (Sphingidae), were reared on artificial diets at 25 °C under a long-day photoperiod (LD 16:8)[6,20]. Larvae of the silkworm, *Bombyx mori* (Bombycidae), a F_1 hybrid strain resulting from a cross between strains J.124 and C.124, were reared on fresh mulberry leaves at 25 °C under constant light.