NEUROENDOCRINE MODULATION OF CARBOHYDRATE METABOLISM

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ABSTRACT

This review discusses the endocrine regulation of carbohydrate metabolism in insects, with special emphasis on the control of trehalose. It also deals with insect hormones involved in the regulation and activity of their mammalian counterparts, insulin and glucagon.

A little over 20 years ago, Steele (1961) reported the presence of a hyperglycemic hormone in the corpora cardiaca (CC) of the American cockroach. He found that 30 min after the injection of an extract equivalent to 1/10 of a gland pair, the hemolymph trehalose had risen by nearly 50%. It reached a maximum of 2.5 fold the normal level 5 h after injection, stayed at about twice the normal level for ca. 24 h, and returned to normal by 48 h. He also found that the level of reducing sugars, primarily glucose, remained essentially unchanged.

Trehalose, present in insect hemolymph, is composed of two glucose molecules with α,α-1,1 linkage, and therefore it is not a reducing sugar. It is present in nearly all the insect species studied (Wyatt 1967), and is the main circulating sugar in insects. The primary, if not the only, site of trehalose synthesis is the fat body. The synthesis of this sugar is summarized in Fig. 1. It involves the formation of trehalose 6-phosphate (T-6-P) from uridine diphosphate glucose (UDPG) and glucose 6-phosphate (G-6-P), and the subsequent hydrolysis of T-6-P. Also shown is the closely related glycogen metabolism. The synthesis of both trehalose and glycogen utilizes UDPG. Therefore, it is important to bear in mind that the kinetics of T-6-P synthase and glycogen synthase may play a crucial role in regulating the synthesis of these two carbohydrates (Murphy and Wyatt 1965). Since trehalose is important to carbohydrate metabolism and appears to be under endocrine control, it has been a subject of rather intensive investigation in the past decade or so. Relatively little has been done concerning the endocrine control of other carbohydrates.

Since the first report (Steele 1961) of the effects of CC on the hemolymph trehalose level, many other reports have confirmed this observation in other insects. These include cockroaches (Bowers and Friedman 1963, Wiens and Gilbert 1967), locust (Goldsworthy 1969), and blow flies (Friedman 1967, Normann and Duve 1969). As mentioned earlier, trehalose is the predominant circulating sugar, and it can be quantitatively determined by enzymatic assays in the presence of other sugars. In the literature one finds that when trehalose is determined specifically, it is the sugar that rises in the hemolymph when CC extract is injected. In such cases I will refer to the hyperglycemic condition as hypertrehalosemia. The term hyperglycemia will be used to describe other cases in which only the total carbohydrates have been determined with such methods as the anthrone assay.

Unlike the adipokinetic hormone, which is synthesized in the glandular lobe of the CC (Goldsworthy et al. 1972), the hypertrehalosemic hormone appears to be synthesized in the brain, probably in the median neurosecretory cells (Highnam and Goldsworthy 1972). It is stored and possibly processed in the CC for release into the hemolymph. Soon after the discovery of the hypertrehalosemic property of the CC, Steele (1963) found that glycogen declined in the fat body

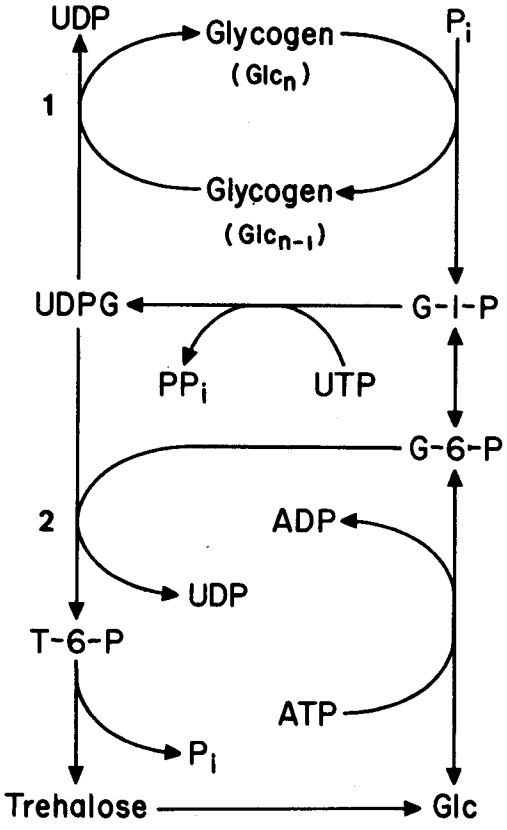

FIG. 1. Biosynthetic pathways of trehalose and glycogen. Enzymes catalyzing reaction 1 and 2 are glycogen synthase and trehalose 6-phosphate synthase, respectively.

of CC-treated cockroaches. Further investigations showed that glycogen phosphorylase activity was increased by CC treatment (Steele 1963). This has also been shown in other insects (Goldsworthy 1970, Wiens and Gilbert 1967). There is evidence that phosphorylase activation in insects is similar to that in the mammalian system, via the "second messenger" (Sutherland et al. 1965). Adenyl cyclase has been found in the fat body of various insects (Morishima 1978, 1979, Filburn and Wyatt 1976, Hanaoka and Takahashi 1977), indicating that this tissue has the capacity to synthesize cyclic AMP. The level of cyclic AMP in the fat body is increased by the CC (Gade 1977, Gade and Holwerda 1976, Gade and Beenakkers 1977, Hanaoka and Takahashi 1977). It is not possible to ascribe the increase in cyclic AMP to the hypertrehalosemic hormone, however, because the CC may contain more than one factor capable of stimulating adenyl cyclase. Cyclic AMP-dependent protein kinases which activate phosphorylase in mammalian systems have been reported in Periplaneta (Takahashi and Hanaoka 1977). Injection of cyclic AMP or its analogs or theophylline, all of which will increase the intracellular levels of cyclic AMP, results in hyperglycemia (Hanaoka and Takahashi 1977). This activation has been observed only in the intact fat body, and there is no evidence of a direct relationship between cyclic AMP and the activation of fat body phosphorylase. Moreover, using rabbit muscle phosphorylase b as a substrate, Ashida and Wyatt (1979) demonstrated that cyclic AMP does not stimulate the phosphorylase kinase isolated from the fat body of Hyalophora cecropia. McClure and Steele (1981) suggested that in the cockroach fat body phosphorylase kinase activity is increased by a rise in intracellular Ca^{2+} levels, which results from the change in Ca^{2+} permeability of the cell membrane caused by cyclic AMP. Therefore, although the evidence for the second messenger system in the insect hypertrehalosemic system is strong, it remains inconclusive. On the basis of this phosphorylase activating property, Steele (1980) has proposed the term "trehalagon" for the hypertrehalosemic hormone after glucagon. This term is not descriptive of the hormone(s)'s structure or function(s), and it implies a single material, which has not been demonstrated. Therefore, the use of such terms should be avoided until more is known about the hormone(s).

Increased phosphorylase alone is not enough to cause a hypertrehalosemic response, because an elevated precursor concentration is not a guarantee of a higher trehalose production. Friedman (1967) demonstrated that CC extracts increased the T-6-P synthase activity of the blow fly fat body in vitro. When the exact mechanism of this process is known, it will help explain the mechanism which causes the elevated trehalose level.

We have shown on a long-term basis that cardiacectomy causes the fat body T-6-P synthase activity to decrease in the blow fly, Phormia regina (Chen and Friedman 1977a). Two days after the surgery the T-6-P synthase activity is only 70% of its normal level. Glycogen synthase, on the other hand, decreases with allatectomy. An attempt was made to reverse the effect of cardiacectomy by incubating T-6-P synthase from cardiacectomized flies with CC extract; this had no effect. If one looks at the incorporation of injected glucose into trehalose in vivo, the effect of cardiacectomy is not apparent unless the insects are forced to fly, that is when they are under stress (Chen and Friedman 1977b). This will be discussed later. Despite the work on the hypertrehalosemic hormone, our knowledge of its mode of action is far from comprehensive, largely because the lack of knowledge of the chemical structure of the active factor(s).

One difficulty in working with insects is the poor availability of sufficient experimental materials. The research on the endocrine control of carbohydrate metabolism is no exception. This is one of the reasons that only limited efforts have been directed toward purifying the hypertrehalosemic hormone. All that can be said is that it is a small peptide because its activity is lost upon trypsin treatment (Migliori Natalizi et al. 1970). Recognizing that the insect hypertrehalosemic hormone bears some resemblance to the mammalian counterpart, namely glucagon, some researchers have resorted to, in my opinion, an essentially backward approach in trying to solve this problem. These investigations have

yielded some controversial but interesting results. Tager and his coworkers (1976), using a radioimmunoassay for bovine glucagon, have identified and isolated a peptide from the CC of the tobacco hornworm, Manduca sexta. It not only cross-reacts with glucagon immunologically, but also displays glycogen-mobilizing activity. Unfortunately a concomitant trehalosemia is not seen. These researchers argue that the tobacco hornworm has a low fat body glycogen content. They calculate that even if all of this glycogen was mobilized it would only increase the hemolymph sugar level by 10%, which is well within the experimental error; therefore, a pronounced hyperglycemia would not be discernible. However, it has been found that injection of neutral red causes a 70% increase in hemolymph trehalose in the same insect (Kramer et al. 1979). This directly contradicts the argument that the tobacco hornworm is incapable of having a hypertrehalosemic response. In addition to this lack of hypertrehalosemic response to glucagon-like material, a fraction from gel filtration chromatography possesses glycogenolytic activity but does not cross-react with bovine glucagon (Tager et al. 1976). Therefore, there might be a glucagon-like material in insects that can cause glycogenolysis, but its identity with the hypertrehalosemic hormone is far from conclusive. Incidentally, Migliori Natalizi et al. (1970) reported the presence of two hyperglycemic hormone fractions in the CC of Periplaneta. It appears that there are multiple forms of hypertrehalosemic hormone. However, one must be cautioned that the hypertrehalosemic hormone(s) and the glycogen-mobilizing hormone(s) may not be the same, because only crude homogenates have been used in most investigations. The two functions have not been ascribed to the same fraction when partially purified hormones are used.

In higher animals the blood sugar level is regulated by two groups of antagonizing hormones - glucagon, glucocorticoids, and epinephrine on the one hand, and insulin on the other. One would think that an analogy might exist in insects. But for more than 10 years after the discovery of the hypertrehalosemic hormone scientists did not concern themselves with this possibility. In the mid 70's, two laboratories independently discovered a hypotrehalosemic hormone in two species of blow flies. During the examination of the long-term effects of cardiacectomy on T-6-P synthase, we determined the trehalose level in cardiacectomized Phormia regina. Much to our astonishment, the hemolymph trehalose in cardiacectomized flies was elevated (Chen and Friedman 1977b). This was contrary to what one would expect when the source of the hypertrehalosemic hormone was removed, especially in light of a lower T-6-P synthase level. We began to wonder if a hypotrehalosemic hormone was present and if in removing the CC we somehow removed this hormone also. In the absence of both hormones, trehalose somehow accumulates in the hemolymph.

We designed a trehalose tolerance bioassay in which the hemolymph trehalose is artificially elevated by injecting trehalose and the rate at which it returns to normal is determined (Chen and Friedman 1977b). In sham-operated insects trehalose quickly returned to normal but when the CC was removed this process was much retarded. Allatectomy had no effects. However, severance of nerves either anterior or posterior to the CC-CA complex gave results similar to cardiacectomy. These results indicate that there is a hypotrehalosemic hormone and that release of the hormone is prevented by the transection of the nerves posterior to the CC. This suggests that either the hormone is in the CC and its release requires input from the brain through the recurrent nerve, or that the hormone is in the brain and transection of the recurrent nerve prevents its release. We decided to explore the CC as the possible source of the hormone. Using cardiacectomized flies with artificially elevated hemolymph trehalose, we found that the CC homogenate had no effects on the decline of trehalose. However, injection of the brain homogenate (3/4 of a brain per animal) restored the cardiacectomized flies' ability to bring down their high hemolymph trehalose level.

It appears that the hypotrehalosemic hormone is more important in carbohydrate homeostasis. Normally trehalose is overproduced, but the hormone helps to

maintain a certain circulating level. In its absence, even when the hypertrehalosemic hormone is absent, trehalose accumulates. The effect of hypertrehalosemic hormone becomes evident only under stressed conditions such as forced flight by stimulating the output of trehalose from the fat body from its glycogen reserves and/or from glucose. Thus when forced to fly, the control flies are able to synthesize trehalose from glucose more rapidly than cardiacectomized flies.

Normann (1975) reported a similar finding in another blow fly, Calliphora erythrocephala, when he followed up the observation that flies surviving decapitation for 30 h had a hypertrehalosemia. He took it a step further and identified the median neurosecretory cells of the brain as the source of the hypotrehalosemic hormone.

Practically nothing is known about the chemical make-up of the hypotrehalosemic hormone, but its neurosecretory origin indicates that it might be a peptide.

To understand the hypotrehalosemic hormone, researchers have again exploited the analogy between insects and mammals. Normann (1975) showed that bovine insulin causes hemolymph trehalose in hypertrehalosemic flies to return to normal. He cautioned against drawing premature conclusions from this result. Tager and coworkers (1976) isolated an insulin-like peptide from the CC of the tobacco hornworm with a bovine insulin radioimmunoassay. Interestingly, this hormone caused a drop in hemolymph trehalose and an increase in fat body glycogen content.

Duve and coworkers (1979) isolated an insulin-like peptide from the head of a blow fly, Calliphora vomitoria, again with a bovine insulin radioimmunoassay. After gel filtration and ion exchange chromatography they obtained a peptide that was judged pure by electrophoresis. It co-migrated with bovine insulin on polyacrylamide gel electrophoresis. When injected into flies made hypertrehalosemic by neurosecretory cell extirpation, this material caused the trehalose to return to normal within 30 min. Similar results have been obtained from Manduca and the honey bee royal jelly (Kramer et al. 1982). Again, it should be pointed out that identifying an insulin-like material in connection with the hypotrehalosemic activity in the insect does not prove that this is the hypotrehalosemic hormone, especially in light of the pharmacological amounts of the insulin-like material used to elicit the hypotrehalosemic response in these studies.

There does appear to be an analogy in carbohydrate metabolism between insects and mammals; that is, they all have antagonizing hormones for the control of circulating sugars. The mechanisms through which these hormones work in mammals have been elucidated, but our understanding of such mechanisms in insects is very rudimentary. More information is required in order to develop selective pest control agents targeted at these systems.

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