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Cephalic Phase, Reflex Insulin Secretion

Neuroanatomical and Physiological Characterization

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Summary. Using chronically catheterized, freely moving male Wistar rats, we have shown that the sweet taste of a saccharin solution reliably triggers a rapid cephalic phase insulin response (CPIR), in the absence of any significant change of glycemia. To establish the neural mediation of this reflex response we used rats that were cured from streptozotocin diabetes by intrahepatic islet-transplantation as a denervated B-cell preparation. The complete lack of any saccharin-induced CPIR in these rats suggests that it is indeed mediated by the peripheral autonomic nervous system, and that the insulin-stimulating gastrointestinal hormones are not involved in this response. It was further found that this reflex insulin secretion is not easily extinguishable and thus might have an unconditioned component. To investigate the central neural pathways involved in this reflex response we used both electrophysiological methods in anesthetized and semi-micro CNS manipulations in freely moving rats. On the basis of our preliminary results, and several reports, using the decerebrate rat preparation for measuring behavioral or saliva secretory oral taste reactivity, it appears that CPIR might be organized at the brain stem/midbrain level, receiving strong modulatory influences from the diencephalon. But much further work has to be done to establish the central nervous circuitry. Finally, in two experiments, aiming at the question of how important and physiologically relevant the CPIR might be, we found that, on one hand, its lack can result in pathological oral glucose tolerance and on the other hand its exaggeration might contribute to the behavioral reaction to highly palatable sweet food and the resulting development of dietary obesity.

Key words: Preabsorptive insulin secretion, cephalic phase insulin response, taste reactivity, B-cell denervation, hepatic islet transplantation, brain stem, diencephalon, hypothalamus, nucleus of solitary tract, glucose tolerance, dietary obesity.

In an excellent recent review, Powley [35] described cephalic phase responses of ingestion and digestion as "autonomic and endocrine reflexes involved in the metabolism of food, that are triggered by sensory contact with foodstuffs rather than by postingestional consequences", and listed as the three defining characteristics a) an afferent limb, consisting of olfactory, visual, gustatory and oropharyngeal mechanical receptors in the head (= cephalic), b) a central nervous system integratory circuit, and c) an efferent limb consisting of a direct neural or a neurally mediated humoral activation of gastrointestinal or visceral target organs. As an example, the secretion of saliva and gastric juices triggered by the taste and smell of food [33] are well known cephalic reflexes.

Cephalic phase insulin responses were first demonstrated in humans, using visual and olfactory stimuli [32] and in dogs, using gustatory and other oropharyngeal stimulation [18].

Three reasons prompted us to investigate cephalic phase insulin responses further. First, because insulin plays a key role in metabolism and body energy homeostasis; second, because cephalic phase input signals are the only known physiological stimuli that produce direct neurally mediated insulin release; and third, because the sweet taste of the food in some societies, seems to be at least partly responsible for one type of human obesity. A valid experimental animal model might therefore be useful.

Demonstration of Cephalic Phase Insulin Response

As discussed by Powley [35], the most straightforward method to measure cephalic phase responses is to prevent the gastrointestinal and postabsorptive phases of food ingestion with the use of an oesophagal fistula. This true sham-feeding has been used successfully in dogs to demonstrate a cephalic phase insulin response [18]. A non-surgical technique has been used in rats by offering non-caloric food [46]. This method eliminates postabsorptive

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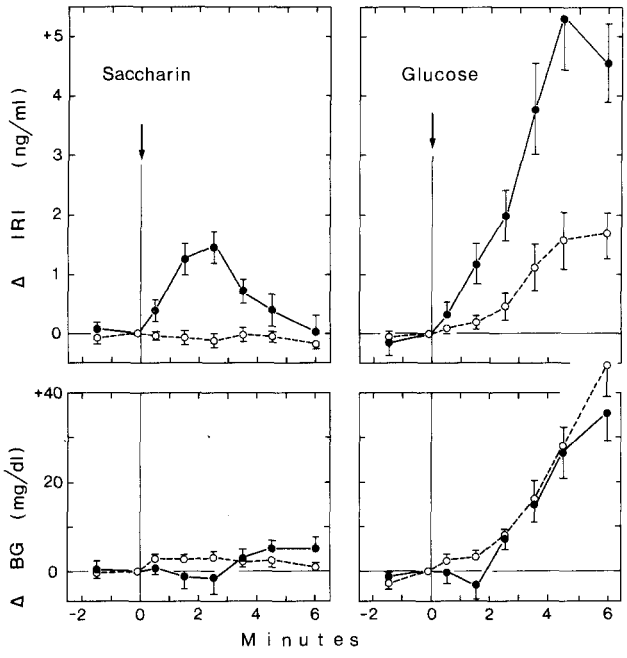

Fig. 1. Changes of peripheral plasma levels of insulin (IRI) and glucose (BG) after ingestion of 1 ml of 0.15% Na-Saccharin or 1 ml of 50% glucose in intact (●—●, n = 6), and islet transplanted rats (o-----o, n = 8). Mean ± SEM. Peripheral venous blood was pumped at a rate of 200 µl/min via chronic jugular catheter into a fraction collector. IRI-baselines for saccharin tests: 1.95 and 1.91 ng/ml, for glucose tests: 2.14 and 1.37 ng/ml, for "Intacts" and "Transplants", respectively. Plasma glucose baselines for saccharin tests: 115 and 123 mg/dl, for glucose tests: 116 and 119 mg/dl, for "Intacts" and "Transplants", respectively

effects, but food still reaches the GI-tract and can stimulate the release of gastrointestinal hormones, which in turn potentiate insulin secretion [1]. Finally, the temporal separation between the early rises of insulinemia and glycemia following ingestion of glucose solutions has also been used to demonstrate that the early insulin rise is cephalically stimulated [24, 25]. Because of the rapid absorption of glucose in the rat [42], this latter method does not allow quantification of the insulin response.

We have therefore chosen the technique of using the ingestion of a saccharin solution as the oropharyngeal stimulus in freely moving rats [6]. Saccharin is preferred by rats to normal tap water and is readily ingested by non food-deprived rats (see [28] for review), but it has no known postabsorptive effects on insulin secretion. By limiting the volume to 1 ml, stimulatory effects on gastrointestinal hormones are very unlikely [34]. Figure 1 shows the mean effect of ingestion of a 0.15% saccharin solution on insulinemia and glycemia in six normal rats (filled circles). The saccharin was ingested within 30–60 sec, causing a 100% increase of insulinemia from baseline, peaking in the second or third post-stimulus minute, and returning to baseline after 5 min. The changes in glycaemia were small and insignificant throughout the test. For comparison the effect of ingestion of 1 ml of 50% glucose is also shown in Figure 1, which demonstrates the rapid increase in glycemia, probably due to early absorption, and considerably higher insulin levels attained by 4 to 6 min as compared to the peak increase with only cephalic stimulation. This saccharin-induced insulin response appears to be relatively small, but one has to consider that it is measured in peripheral venous blood and is an attenuated reflection of the response in the portal vein, with up to 50% of released insulin extracted by the liver [27]. The remaining hormone levels are further diluted by the extrahepatic venous blood. In dogs, the increase in insulinemia was approximately 5 times higher in portal blood than in peripheral blood five minutes after ingestion of 1 g/kg glucose [19].

To demonstrate definitively that insulin release following saccharin ingestion is a direct neural phenomenon between oropharyngeal sensory input and autonomic efferent nerve output to the pancreatic islets, control experiments are needed which: 1) show that interruption of a presumed pathway of the reflex loop abolishes the response, and 2) specify the type and location of the receptors at the origin of the reflex.

Concerning the first point, it is important to demonstrate that interruption of the efferent autonomic neural outflow to the islet abolishes the insulin response, because it is difficult to control all the non-neural influences on the B-cells in a freely moving rat. To this end the following manipulations have been successfully utilized: muscarinic cholinergic blockade by atropine [12], subdiaphragmatic vagotomy [11, 24, 25], complete surgical pancreatic denervation [12], and selective denervation of the B-cells by means of transplantation of either foetal pancreases underneath the kidney capsule [24], or pancreatic islets into the liver [49] in rats whose own B-cells have been previously destroyed with streptozotocin. The more definitive measure might be the last mentioned, since it selectively denervates the B-cells while leaving the innervation of other islet cells, and of the gastrointestinal tract intact. We injected about 2000 isolated islets into the portal vein of a streptozotocin-treated diabetic recipient rat, representing approximately 60% of the pancreatic insulin content of rats of this strain and age [49]. Within two weeks after islet transplantation, all the gross signs of diabetes, including increased urine volume, increased glycemia and decreased body weight gain, were

reversed to normal. By 10 weeks after transplantation, when the body weights were identical for the transplant and sham-operated control groups, testing was begun. It was first seen that fasting plasma levels of glucose and IRI, and intravenous glucose tolerance were normal in the transplants in spite of an insulin output which was lower than that of controls. Considering that only about 60% of the normal pancreatic insulin content was transplanted, it appears that the grafted B-cells are able to respond rapidly and are normally sensitive to intravenous glucose. In contrast, Figure 1 (broken lines) shows that the saccharin stimulated insulin response normally seen in intact rats is completely absent in the transplanted animals. This finding is strong evidence that the abolition is due to an interruption of the neural supply to the grafted islets, although the decreased insulin output following pure substrate stimulation (intravenous glucose) probably also contributes. These results also suggest that the entero-insular axis does not participate in the saccharin induced insulin response, assuming that the gastrointestinal hormones reach the B-cells in their new environment as easily as in control rats.

Concerning the second point for definition, to specify the nature of the receptor involved at the origin of the reflex, we have tested, besides saccharin and glucose, tap water as a neutral gustatory stimulus, and distilled water or quinine as aversive stimuli. We found that all of these stimuli were able to produce positive insulin responses in certain animals and under certain experimental conditions. However, as shown in Figure 2, the mean insulin response to tap water or quinine was considerably smaller than the response to saccharin. It is difficult to understand why the taste of quinine or distilled water (neither of them was ingested, but they were tasted repeatedly) would produce a small insulin response. However, cephalic phase salivary flow in response to quinine has been demonstrated and parallels the increase in chorda tympani afferent nerve activity [21]. This small rise in insulin secretion following quinine may reflect a general activation of gustatory afferent receptors, only some of which activate appropriate secondary neurons that are linked to efferent autonomic fibers facilitatory for insulin release. It is unlikely to be simply an artefact of the transition from a resting to an active state of the animal, since motor activity tends to decrease insulinemia [50]. Another possible explanation is that the insulin response has become conditioned to nonspecific stimuli associated with the presentation of the test solution, like lifting the lid of the cage, etc. We have tested this hypothesis by presenting distilled water three times within one session, and found that

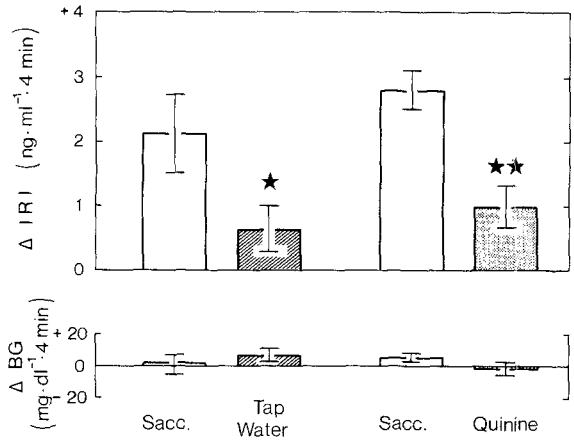

Fig. 2. Mean cephalic phase response amplitudes expressed as integrated, incremental IRI- and glucose areas following non-sweet control stimuli: tap water (n = 9) and 0.05% Quinine hydrochloride (n = 12). Two separate groups of rats. Statistical comparisons with paired t-Test, *p <0.05, **p<0.001

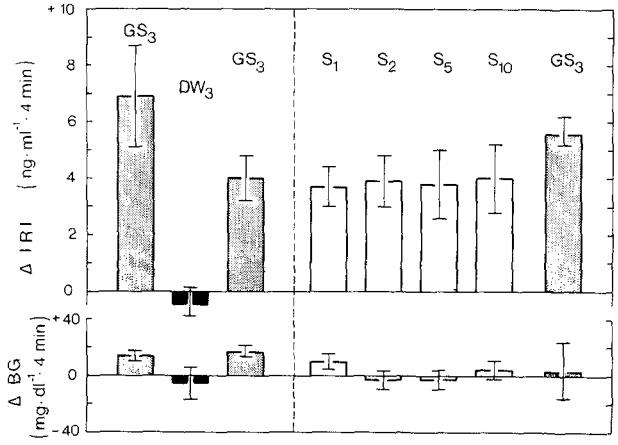

Fig. 3. Cephalic phase insulin response-amplitudes and corresponding glucose changes following different oral stimuli of 3 rats (Mean ± SEM). In the initial phase of the experiment response-amplitudes were twice measured following the third consequetive presentation of glucose + saccharin, GS_3 or distilled water, DW_3. S_1–S_{10} represent the attempted extinction phase of the experiment. S_1 and S_2 as well as S_5 and S_6 were separated by one day of normal feeding, the other extinction trials were performed with intervals of one hour on the same day. At the end of the experiment glucose + saccharin was given again as a control

following the third presentation there was no insulin response (Fig. 3, DW_3). This finding is consistent with the notion of the distilled water response being conditioned, since it follows the laws of extinction. This leads us to the important question as to whether the saccharin-induced insulin response itself, or part of it, is conditioned or not. Saccharin, unlike glucose ingestion, does not create a postabsorptive state

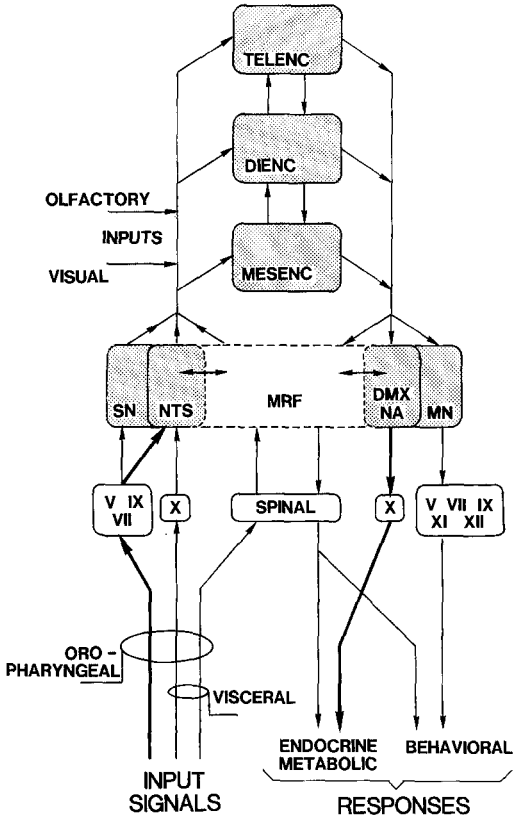

Fig. 4. Highly simplified, schematic, neural flow diagram, demonstrating the relationship between the afferent limb (input signals), the possible central integrative network, and the efferent limb (responses) of reflexes of ingestion and digestion. The possible pathways of the cephalic phase insulin reflex are indicated with heavy lines. V–XII = cranial nerves, DMX = dorsal motor nucleus of the vagus, MN = motor nuclei of other cranial nerves, MRF = medullary reticular formation, NA = nucleus ambiguus, NTS = nucleus of the solitary tract, SN = other sensory nuclei of the brain stem

which is associated with insulin secretion, and could be considered as a conditioned stimulus to signal to the animal ingestion of glucose [7, 9]. If this is so, repeated stimulation with saccharin should lead to extinction of the observed insulin response [9]. As illustrated by Figure 3, this is not the case even after the tenth saccharin presentation (S_{10}), suggesting that the sweet taste-induced, neurally-mediated insulin response is an unconditioned reflex. This finding is supported by the observations that saccharine preference also does not extinguish [40], (see also the discussion of [28]), and that rats adapted to carbohydrate free food for two weeks still display a large cephalic phase insulin response to it [46].

To summarize briefly, sweet taste and possibly other cephalic sensory inputs, have been identified as unconditioned insulin releasing stimuli. The reflex

Table 1. Diencephalic stimulation sites[a] evoking unit activity in chorda tympani-responsive neurons in the nucleus of the solitary tract

	Neurons tested	Neurons responsive
Anterior hypothalamus	24	0
Medial hypothalamus	19	0
Lateral hypothalamus	70	38
Optic tract and supraoptic commissure	12	0

[a] Diencephalic stimulation consisted of biphasic rectangular pulses, 0.2 msec pulse duration, and current intensities of 200–500 μA, applied via a bipolar concentric stainless steel electrode

loop comprises oral taste receptors and most probably parasympathetic vagal efferent fibers with their peripheral muscarinic cholinergic receptors to the pancreatic B-cells. Obviously, the two limbs are integrated by the central nervous system.

Underlying Central Neural Pathways

Gustatory inputs have their first relay in the caudal brain stem and then reach other levels of the brain, like midbrain, hypothalamus, the thalamic taste area and the gustatory cortex [31] (see also Fig. 4). Existing data on gustatory-evoked secretory and behavioral responses suggest that a high degree of integration may reside at the brain stem and/or midbrain level and that the hypothalamus and other diencephalic and telencephalic structures are not essential for the basic reflex, although they are important modulators.

First, Kawamura and Yamamoto [21] have shown that salivary secretion is well correlated with chorda tympani afferent nerve activity in anesthetized, decerebrate rabbits and is quite sensitive to the gustatory qualities of the applied oral stimuli. They concluded that the basic acceptance/rejection reflex for oral ingestion occurs at the brain stem level. This conclusion was essentially corroborated by the behavioral studies of Grill and Norgren [14], where they demonstrated that decerebrate rats display the same stereotypical pattern of acceptance or rejection to appropriate oral test stimuli as that seen in control animals. Kornblith and Hall [22] have shown that the ingestive response of neonatal rats is not disrupted by decerebration, but is severely altered after diencephalic transections. Also, anencephalic human infants display normal gustatory-evoked facial expressions [44]. Finally, decerebrate rats are even capable of showing a certain degree of satiety [15].

Furthermore, recent anatomical studies [38] have shown that hypothalamic neurons innervate brain stem regions which receive afferent gustatory information (nucleus of the solitary tract = NST) as well as brain stem nuclei which contribute efferent fibers to the vagal nerve (dorsal motor nucleus of the vagus = DMX, and nucleus ambiguus = NA, Fig. 4).

We have extended these anatomical findings using electrophysiological methods [3]. Chorda tympani-responsive NTS neurons were first identified by electrically stimulating an isolated portion of the peripheral chorda tympani nerve as it emerges from the skull. Such electrical stimuli evoked single unit discharges in secondary NTS neurons assumed to be gustatory-sensitive. The activity of these identified neurons was then tested for responsitivity to hypothalamic stimulation. As shown in Table 1, many chorda tympani-sensitive neurons could also be driven by lateral hypothalamic electrical stimulation. Effective hypothalamic stimulation points were confined to the lateral hypothalamic area. Medial hypothalamic stimulation never activated chorda tympani-sensitive brain stem units. These data demonstrate that hypothalamic sites previously implicated in insulin release and feeding behavior can modify the activity of brain stem units receiving oropharyngeal sensory input.

More recently we have found that vagal-sensitive neurons in the region of DMX and NA are also activated by chorda tympani stimulation (data not shown). This latter point deserves some mention as it suggests that gustatory-evoked vagal activity, such as that proposed to underlie the cephalic phase reflex release of insulin secretion, may be organized to a certain degree strictly at the brain stem level. Hypothalamic input may simply be modulatory and not obligatory for cephalic phase reflex responses.

Of particular relevance to this discussion is the potential role of the ventromedial hypothalamus (VMH) in modulating cephalic phase reflexes. Since VMH-lesioned animals display dramatic alterations in food preference [48], it has been postulated [35] that such lesions may modulate cephalic phase reflexes including the early insulin release following food ingestion. In support of this hypothesis, elevated preabsorptive insulin secretion has been reported in VMH-lesioned rats after they have become overtly obese and hyperinsulinemic [24]. It was not clear from these preliminary data whether the elevated cephalic insulin response was due to a real exaggeration of the amplitude of the reflex or to an hypertrophied B-cell mass as is found in chronically VMH-lesioned rats [16, 17, 37]. To avoid such difficulties of interpretation, we measured the saccharin-induced cephalic phase insulin response in the presence or absence of acute reversible procaine treatment of the VMH in freely moving rats [4]. Since procaine microinjection into the VMH elicited feeding, it was assumed that this procedure allows us to mimic repeatedly and reversibly the behavioral state typically seen after VMH lesions [10]. Rats were chronically implanted with bilateral VMH guide cannulas and a jugular catheter for blood sampling. Only animals which reliably ate in response to procaine injections were used for measurement of the saccharin-induced cephalic phase insulin response. The data shown in Table 2 demonstrate that prior VMH procainization attenuated rather than increased the early insulin response, the attenuation being dose dependent. These data do not directly support Powley's [35] cephalic phase theory of the VMH lesion syndrome, but alternatively, one can postulate that VMH procaine injections and VMH electrolytic lesions are not, in fact, equivalent methods to block VMH neuronal function. From a purely neurostructural point of view, this is certainly true. While electrolytic VMH lesions destroy all neuronal elements within their focus, there is evidence that procaine might selectively suppress different neuron populations [13]. Since feeding can be induced by electrical [8] or chemical [5] stimulation of the ventromedial hypothalamus, we suggest that the present procaine injections may selectively activate an equivalent neuronal population either by preferentially blocking local inhibitory interneurons or by interrupting short axon fiber conduction (intrahypothalamic?) rather than longer fibers of passage. The fact that cephalic phase insulin release was significantly reduced rather

Table 2. The effect of prior intrahypothalamic procaine injection on amplitude of subsequent saccharin-induced cephalic phase insulin response

Integrated[a] incremental area	VMH-Pretreatment[b]		
	Saline	Procaine-HCL	
		2 × 15 µg	2 × 50 µg
Insulin ($ng \cdot ml^{-1} \cdot 4\,min$)	5.56± 1.64	2.19±0.62	−1.07±1.09[c,d]
Glucose ($mg \cdot dl^{-1} \cdot 4\,min$)	8.5 ±11.1	−4.5 ±4.0	8.9 ±5.5[d]

[a] Mean ± SEM of 4 rats

[b] The substances were injected bilaterally in 1 µl volumes 4 min. before the oral saccharin-ingestion. The rats included in this analysis were selected on the basis of their acute feeding behaviour in response to the higher procaine dose, and on their significant cephalic insulin response during VMH-saline treatment

[c] Significantly different ($p<0.02$) vs saline control

[d] Significantly different ($p<0.05$) vs 2 × 15 µg procaine

Table 3. Increase of body weight gain, baseline body weight gain and IRI, and initial body weight of cephalic phase "low" and "high" responders

Group[a]	IRI baseline	Saccharin induced cephalic phase IRI-response	Initial body-weight	Body weight gain[b]	
				Pre-diet	Increase on diet
	ng/ml	$ng \cdot ml^{-1} \cdot 4\,min$	g	g/day	g/day
Low (n=11)	3.04[c] ±0.19	+2.76±0.45	479 ± 10	2.88 ±0.27	2.54 ±0.46
	n.s.	$p<0.05$	n.s.	n.s.	$p<0.001$
High (n=11)	2.97 ±0.16	+4.48±0.56	478 ± 10	2.35 ±0.27	4.19 ±0.32

[a] The rats were arbitrarily divided in two groups on the basis of their sweet-specific cephalic insulin response, which was obtained by substracting the quinine-induced non-specific response from the saccharin-induced response for each individual rat
[b] Daily body weight gains were calculated on the basis of two eight day periods, one just prior and the other during a "cafeteria-diet", consisting of 4 items of highly palatable sweet food, freshly offered in the morning and late afternoon
[c] Means ± SEM, unpaired t-Test for comparisons

Table 4. Integrated incremental insulin- and glucose areas during 1 g/kg oral GTT in intact and islet transplanted rats with or without an exogenous insulin injection at the beginning of the test

Integrated[c] incremental areas	Intact rats	Transplants[a]	
		Without insulin injection	With insulin[b] injection
IRI 0–5 min ($ng \cdot ml^{-1} \cdot min$)	12.9 ±3.0	2.4[d] ±1.0	14.2[e] ±1.9
IRI 0–60 min ($ng \cdot m^{-1} \cdot min$)	291 ± 37	140[d] ± 20	131[d] ± 28
Glucose 0–60 min ($mg \cdot dl^{-1} \cdot min$)	1'315 ±102	5'087[d] ±1003	2'420[e] ±743

[a] Endogenous B-cells were destroyed by streptozotocin, and the resulting diabetes treated by injection of 2000 islets per rat into the portal vein. Resting levels of glycemia and insulinemia as well as urine volume were normalized. At the beginning of the testing period, 12 weeks after transplantation, intravenous glucose tolerance (1 g/kg) and body weight were not significantly different for the two groups
[b] Actrapid (NOVO) insulin was injected intravenously one minute after the start of glucose ingestion, in a dose to mimic the concentration found in peripheral veins during cephalic phase release
[c] Means ± SEM of 6 animals in each group
[d] $p<0.01$ vs. intact rats
[e] $p<0.01$ vs. transplants without insulin injection

than exaggerated by VMH procaine injections is not disturbing. What is of primary importance is that cephalic phase reflex release of insulin can be modulated by VMH manipulation and be uncoupled from the evoked feeding behavior, as electrolytic lesions exaggerate the response while procaine injections attenuate insulin release relative to control.

In summary, there is considerable evidence supporting the view that at least some gustatory-evoked secretory and ingestive behavioral responses are organized at the brain stem level. Whether the gustatory-evoked, cephalic phase insulin reflex is similarly organized at this level has not yet been directly tested. Furthermore, we presented both anatomical and functional support for the concept that diencephalic structures such as the hypothalamus are able to modulate gustatory-evoked cephalic responses. However, much has to be done to identify further the critical central nervous system pathways.

Possible Physiological Significance of Cephalic Phase Insulin Response

Elevated basal and stimulated insulin levels are intimately related to the occurrence of obesity [23, 24, 36, 45]. Obesity per se is accompanied by increased basal insulin levels and decreased oral glucose tolerance in man [41], and experimentally-induced exaggerated insulin levels can cause the development of obesity (see [2], for a review). With respect to cephalic phase insulin secretion, it was found that formerly obese people who lost weight on a regimen and whose basal insulinemia had returned to the levels of the non-obese control group, still displayed a larger visual and olfactory-evoked cephalic phase insulin response [36]. We designed an experiment in which the amplitude of the saccharin-induced cephalic phase insulin secretion was measured in individual rats before they became obese. Using a so-called "cafeteria" or "supermarket" diet [20, 39], body weight gain above baseline during the first 8 days was taken as a measure of the degree of obesity developed by a particular rat. The experimental

paradigm was based on observations that the individual variation of both the amplitude of cephalic insulin responses ([18, 32], and personal observations) and the supermarket diet-induced body weight gain [39] are large. The question could therefore be asked if rats displaying high amplitude cephalic phase insulin responses would be more likely to become obese on highly palatable and varied food than rats displaying low insulin responses. The animals were thus divided into two groups according to the magnitude of their saccharin-induced insulin response. It can be seen from Table 3 that the "high" cephalic phase responders increased their body weight gain significantly more during the diet than did the "low"-responders. The mean insulin baseline levels, body weight, and pre-diet body weight gain were similar for the two groups. This finding supports the concept of a causative relationship between the amplitude of orosensory-endocrine-metabolic reflexes, appetite, and likelyhood to develop obesity in the rat. Note, however, that a small cephalic phase insulin response does not abolish the hyperphagic effect of a palatable diet. Nevertheless the model has a striking similarity to what could be considered the most common type of human obesity, which is the constant exposure to highly palatable food in some western societies [26].

As we have seen above, rats with denervated B-cells lack a cephalic phase insulin response. Interestingly in the subsequent later phase of an oral glucose tolerance test, blood glucose stays elevated longer, amounting to a decreased glucose tolerance [25, 49]. Furthermore, if the cephalic phase is bypassed using intragastric glucose [25] or high carbohydrate liquid food loads [43], postabsorptive glucose levels are significantly higher than after oral loads. These facts suggested that the cephalic phase insulin secretion might play an important role in the disposal of oral glucose loads. To test this hypothesis, the missing cephalic phase insulin response of islet-transplanted rats was mimicked by intravenous insulin injection. Table 4 shows that this injection increased the 5 min.-integrated incremental insulin area to a value which was no longer significantly different from the one obtained in intact rats, while it did not affect the total insulin area over the whole length of the test. By contrast, this manipulation significantly reduced the 60 min. glucose area by 50%, further suggesting that the lack of cephalic phase insulin secretion is important for normal oral glucose tolerance.

As others have suggested [29], the cephalic phase insulin response and other cephalic responses (gastric acid secretion, exocrine pancreatic secretion, etc.) prepare or prime the mechanisms that will soon have to transport, break down, and store incoming nutrients. Insulin is important, since it promotes glycogen synthesis and triglyceride production in the liver, and safeguards the upper limit of glycemia. The liver, besides being an indirect target, is probably also a direct target organ of cephalically triggered neural signals, although cephalic phase hepatic responses have not yet been demonstrated. On the other hand, cephalic phase endocrine pancreatic release of glucagon [30] and pancreatic polypeptide [47] have been demonstrated, but their physiological role is not yet understood.

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Discussion after Berthoud's Presentation

Woods: I think that your conclusion regarding the apparent unconditioned or hardwired nature of the cephalic insulin response may be somewhat premature, especially when it is based on the lack of extinction over 10 trials. In the only other study on this topic of which I am aware, Deutsch, using different parameters, did find apparent extinction (J Comp Physiol Psychol (1974) 86: 350). Without knowing the conditioning history of your rats, it might be unreasonable to expect extinction in so few trials; and it is certainly the case that whether the response is unconditioned or not, it is very modifiable through conditioning.

Berthoud: I agree, but I'm not sure that Deutsch's paper is relevant since he measured hypoglycemia at 2 h after the saccharin was sampled.

Nicolaidis: Many years ago, we did an experiment in which saccharin was put on the tongue of anesthetized rats and they responded with a lowering of blood glucose. There was certainly no learning operating in that study. On the other hand, in a more recent study in rats were chronically fed with continued intravenous infusions of glucose, we have been able to increase the anabolic efficiency of infusates by allowing them to consume a saccharine solution. This effect, attributed to a reflexly released supplement of endogenous insulin, was attenuated after one or two days. I expect that you are both correct and that there is a small unconditioned component which is very susceptible to modification by experience.

Berthoud: I agree, and I must admit that in my experiment, the rats were allowed to eat food after the extinction trials began. These meals might be considered as reinforcement trials.

Novin: In fact, those intermittent feedings are probably counterproductive in terms of conditioning because the animals were getting mixed trials (some reinforced and some not) and probably becoming very resistant to extinction.

Porte: When Pipeleers made his islet transplants into the portal circulation as you have done, he found that the islets were supersensitive to catecholamines (Diabetes (1978) 27: 817). It is now pretty clear that when animals eat, there is a considerable increase of catecholamines. Perhaps the apparent lack of a cephalic insulin response you see in your rats is at least partially caused by an exaggerated suppression of insulin secretion by these catecholamines. Have you ever looked at catecholamine levels in your animals?

Berthoud: No, we haven't looked at catecholamines and so I cannot comment on that possibility.

Steffens: As you know, we have studied the insulin response which occurs when nor-epinephrine is administered into the lateral hypothalamus. We suspected a direct neural link to the pancreas since the response is eliminated with vagotomy. However, we unexpectedly found that the response is actually increased in islet-transplanted rats, suggesting perhaps a supersensitivity to some humoral agent.

Nicolaidis: Does anyone know about possible reinnervation of transplanted islets?

Bray: I think it's pretty clear that as vessels grow into the transplanted tissue, sympathetic axons grow in with them. In the liver, which has a rich vagal innervation, perhaps vagal fibers innervate the transplanted islets as well.

Berthoud: There is one study which clearly showed nerve terminals in transplanted islets (Diabetes (1977) 26: 201), but contact with the cells has never been established.

Porte: Actual contact is probably not important; proximity may be.