Insulin interaction with the central nervous system: nature and possible significance

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The endocrine and nervous systems are the two major integrators of mammalian physiology. The central nervous system (CNS) has been recognized to control certain metabolic processes since the observation by Bernard (1849) that puncture of the floor of the fourth ventricle resulted in the appearance of transient glucosuria. Hetherington & Ranson (1939) showed that rats with lesions of the ventromedial hypothalamus developed hyperphagia and obesity; and Hales & Kennedy (1964) showed that these animals were hyperinsulinaemic. In that same year Anand *et al.* (1964) showed that intravenously administered glucose rapidly altered the electrical discharge activity of hypothalamic neurons. Shimazu & Amakawa (1968) demonstrated that hepatic glycogen metabolism is regulated by the autonomic nervous system. Thus it has become evident that changes in metabolite levels influence CNS activity and conversely metabolic factors are influenced by the CNS.

Since 1970, evidence has been accumulating that insulin acts on the CNS. Debons *et al.* (1970) first provided evidence for an effect of insulin on the hypothalamic satiety centre. Szabo & Szabo (1975) then showed that intracarotid insulin injection produced a fall in blood glucose, and this was complemented by the identification (Posner *et al.* 1974) of insulin receptors in crude membranes from brain. The demonstration that direct injection of insulin into the ventromedial nucleus of brain produced hypoglycaemia clearly established a function for insulin receptors within the hypothalamus (Storlein *et al.* 1975).

The circumventricular organs of brain: unique sites for neuroendocrine regulation

The circumventricular organs (CVO) of the brain are aggregates of specialized nervous tissue which interface between the systemic and cerebrospinal circulations (Weindl, 1973). They consist of four midline structures abutting the ventricular lumen: namely, the organum vasculosum of the lamina terminalis (OVLT), located on the rostral aspect of the third ventricle; the subfornical organ (SFO), located at the junction of the third and lateral ventricles; the area of postrema, a wedge of nervous tissue bulging dorsally into the fourth ventricle and intimately associated with sensory and motor nuclei of the vagus; and the median eminence, the proximal segment of the hypophyseal infundibulum which bridges the two halves of the basal hypothalamus.

The CVO possess several distinctive features (Weindl, 1973). They are highly vascular and their capillary endothelial cells are fenestrated, unlike elsewhere in the

CNS where endothelial cells are non-fenestrated and possess tight functions, thus constituting a blood–brain barrier to macromolecular transit (Broadwell & Brightman, 1976). In both the median eminence and OVLT, nerve terminals cluster around the perivascular spaces compatible with the well-recognized neurosecretory role of the former. In the area postrema and SFO, the aggregates of nerve terminals are supplemented to sizeable numbers of neuronal cell bodies.

CNS receptors for blood-borne insulin

The combination of distinctive permeability and concentration of neuronal elements led us to hypothesize that the CVO may be targets for blood-borne peptide hormones and hence an avenue by which metabolic information can be channelled into the CNS. We used a procedure for identifying receptor sites *in situ* (Bergeron & Posner, 1979) in which ^{125}I-labelled insulin was systemically injected followed by perfusion with a fixative solution to retain receptor-bound radioactivity for radioautographic visualization and quantitative analysis of insulin receptors in the CNS. Since the intensity of radioautographic reactions reflects the amount of bound radioactivity (Junger, 1978), the difference in reaction intensities observed following the injection of ^{125}I-labelled insulin alone or with excess unlabelled insulin provides, in analogy with the in vitro determination of specific binding (Posner, 1975), a measure of specific binding sites *in situ*.

Using this approach we have localized specific binding sites for blood-borne insulin to two different compartments of the CNS: (a) the microvessels distributed throughout the CNS; and (b) the CVO of the brain.

Insulin binding to brain microvessels. Following ^{125}I-labelled insulin administration, radiolabel was radioautographically identified in microvessels in all brain regions. The binding was progressively inhibited by increasing concentrations of co-injected unlabelled insulin, but not by structurally dissimilar polypeptides. Electron-microscope radioautography identified the microvessel endothelium as the cellular site of the insulin receptors (van Houten & Posner, 1979). These initial observations on rat brain were subsequently extended to primate brain (Landau *et al.* 1983). Microvessel binding can explain, at least in part, the presence of insulin receptors in homogenates and subcellular fragments from many different brain regions (Havrankova *et al.* 1978; Pacold & Blackard, 1979). Our radioautographic studies have been subsequently confirmed by direct in vitro binding assays on purified brain microvessel preparations (Frank & Pardridge, 1981). The functional significance of these microvessel insulin receptors remains uncertain. They may mediate insulin effects on transport processes across the blood–brain barrier or function as carriers for the transport of insulin (i.e. transcytosis) into brain regions behind this barrier, or both. A precedent for their engagement in transcytosis has been established for prolactin receptors in rat choroid plexus which have been shown to transport prolactin from the systemic circulation into the cerebrospinal fluid.

The hormone-receptor role of CVO. Within 5 min of the systemic injection of ^{125}I-labelled insulin, specific binding sites were readily identified in all the CVO.

Detailed study of the median eminence showed that ^{125}I-labelled insulin binding was blocked by co-injected unlabelled insulin, insulin analogues and structurally dissimilar peptide hormones in parallel with their ability to inhibit insulin binding to its receptors in vitro (van Houten *et al.* 1979). Similar findings were documented for the adjacent arcuate nucleus and the area postrema (van Houten & Posner, 1981). Thus the CVO-binding sites for insulin have the specificity characteristics of biologically important receptors.

Neural basis of specific binding sites in the CVO: mediating mechanisms

How is the specific binding interaction in the CVO transformed into a signal to endocrine, autonomic and behavioural regulatory systems in the CNS? The first step toward answering this question is to identify the cellular sites of peptide hormone binding in the CVO and to determine the anatomic relations of these target cells to the CNS. This has been accomplished using quantitative in vivo electron-microscope radioautography.

In the median eminence, specific binding sites for blood-borne ^{125}I-labelled insulin have been localized to nerve terminals and in the adjacent hypothalamic arcuate nucleus to synaptic terminals (van Houten *et al.* 1980). These axonal receptors may be hypophysiotropic terminals involved in mediating insulin effects on the secretion of growth hormone (Pecile *et al.* 1971) or gonadotropin (Rossi & Bestetti, 1981). In addition, insulin-receptive nerve terminals may play a more complex role in mediating insulin effects on hypothalamic electrical activity (Oomura, 1976), catecholamine turnover (McCaleb *et al.* 1979) and limbic functions related to glucose homeostasis (Storlein *et al.* 1975; Iguchi *et al.* 1981), energy balance and satiety (Hatfield *et al.* 1974; Woods *et al.* 1979).

The neuronal structures to which these axons belong have been recently elucidated by the combined application of selective surgical ablation and radioautography (van Houten *et al.* 1983). These studies have established that insulin-receptive nerve terminals in the median eminence arise from so-called tuberoinfundibular neurons of the hypothalamic arcuate nucleus. Tuberoinfundibular neurons in this region are thought to possess a complexly branched axonal tree that projects into the CNS as well as the median eminence and could permit these neurons to act on the brain and pituitary simultaneously (Renaud & Martin, 1976). Recent studies have shown that blood-borne peptide hormones can electrically activate certain neurosecretory axon terminals (Baertschi *et al.* 1981), and that electrical signals generated at the neurosecretory terminal can be transmitted 'backward' into the brain via the tuberoinfundibular neuron (Renaud & Martin, 1976). We have suggested that a result of insulin binding to its receptor in the median eminence may be the production of an electrical signal that is relayed to specific regulatory centres in the brain via the axonal tree of the receptive tuberoinfundibular neuron (Fig. 1). These peptide-receptive neurons could provide the essential anatomic pathway linking endocrine feedback to the appropriate central integrative systems governing autonomic and behavioural outflow.

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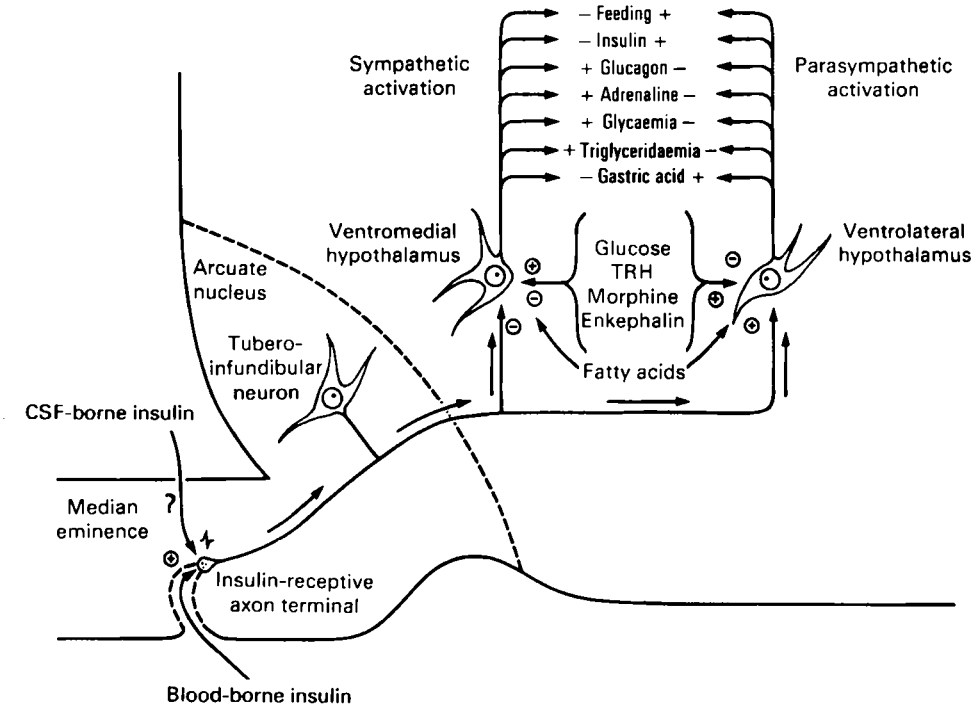

Fig. 1. A postulated role for tuberoinfundibular neurons of the arcuate nucleus in transducing insulin blood levels into a neurophysiologic input influencing both the ventromedial and ventrolateral hypothalamus. Neurons in the ventromedial hypothalamus are inhibited whereas those in the lateral hypothalamus are stimulated by insulin (Oomura, 1976). Stimulation of the ventromedial hypothalamus produces sympathetic activation (Bernardis & Frohman, 1971; Bray & Nishizawa, 1978); whereas stimulation of the ventrolateral hypothalamus produces parasympathetic activation (Shimazu, 1981). The two regions are mutually inhibitory and tend to show reciprocal activation/inhibition with various stimuli (Oomura & Kita, 1981). TRH, thyrotrophin-releasing hormone.

Another possible mechanism of peptide hormone action on the CNS via the CVO is illustrated by the direct binding and internalization of circulating insulin by neuronal cell bodies and dendrites of the rat area postrema (van Houten & Posner, 1981). Neurons in the area postrema contact other neurons in the caudal visceral subdivision of the nucleus solitarius (Morest, 1967), which in turn contact parasympathetic preganglionic neurons in the dorsal motor nucleus of the vagus and sympathetic preganglionic neurons located in the intermediolateral cell column of the thoracic spinal cord (Loewy & Burton, 1978). Thus, insulin-receptive neurons in the area postrema may function to monitor circulating insulin and modulate, accordingly, brainstem autonomic circuits that govern vagal and splanchnic outflows (Fig. 2).

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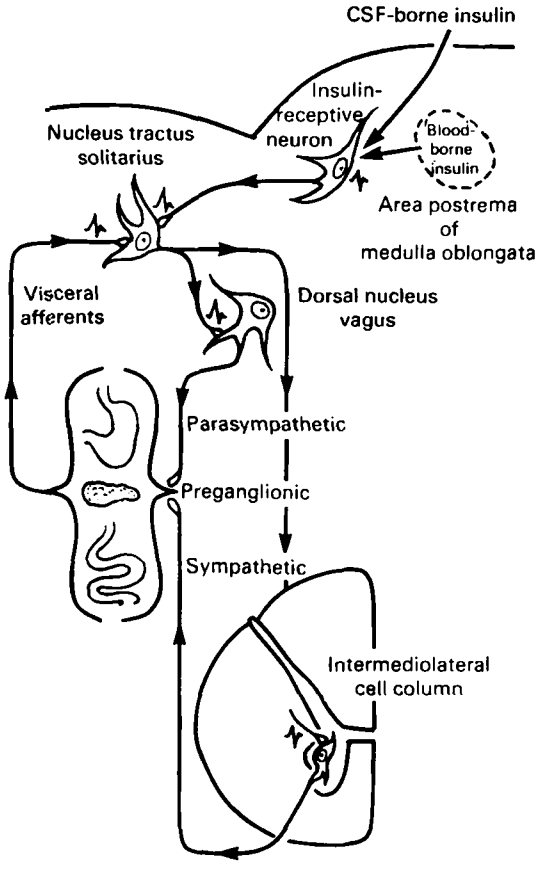

Fig. 2. A proposal as to how insulin, interacting with neurons of the area postrema, can influence autonomic outflow to the gut. The hormone-sensitive neurons in the area postrema are depicted as modifiers of visceral afferent input into the nucleus solitarius (Morest, 1967).

In either case it appears that neuronal circuitry exists to link insulin receptors in the CVO to neurons behind the blood–brain barrier, which determine patterns of behaviour and autonomic function. Similar pathways may exist to mediate the effects of other circulating peptides on brain function (van Houten & Posner, 1983).

Significance

This review emphasizes the relatively novel concept that the CVO mediate the direct feedback effects of insulin on brain function. Comparable observations have been made for a variety of peptide hormones including growth hormone, calcitonin, ACTH and angiotensin (van Houten & Posner, 1983). A particular emphasis has been placed on the possible role of tuberoinfundibular neurons of the

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arcuate nucleus in transducing endocrine into neurophysiologic signals. This conceptual framework will hopefully prove helpful in designing experiments aimed at a full understanding of the neural mechanisms involved in endocrine–CNS communication.

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