Pituitary adenylate cyclase activating peptide (PACAP) in the retinohypothalamic tract phase shifts the circadian clock

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Pituitary adenylate cyclase-activating peptide (PACAP) is highly concentrated in limbic and neuroendocrine areas. It is a stimulator of adenylate cyclase and considered to play a role as a regulator of pituitary function, neuronal transmission, and neurogenesis [1]. A very high level of PACAP-immunoreactivity, but no PACAPmRNA has been detected within the suprachiasmatic nucleus (SCN) of the rat [2]. It was therefore suggested that PACAP is present in one of the major input pathways to the clock and may participate in the regulation of circadian timing. In this review, we will present our work on PACAP-mediated neurotransmission in the SCN. These studies utilise a wide range of techniques, including neuronal tract tracing, immunocytochemistry, in situ hybridisation, and electrophysiology in the SCN brain slice.

1. PACAP in the retinohypothalamic tract

The distribution of PACAP in the SCN was studied by means of immunocytochemistry. Adult male Wistar rats and Syrian hamsters were perfused with a solution of 2.0 % paraformaldehyde and 0.15% picric acid, the brains frozen, sectioned on a cryostat and processed immunocytochemically using a specific monoclonal antibody against PACAP [3].

Several staining procedures and chromagens were applied in these studies [3,4]. In the hamster, a dense accumulation of PACAP-immunoreactive terminals was observed in the ventral SCN (Fig. 1). The distribution of the dense immunoreactive material overlapped with the retinorecipient area of the SCN [5]. This zone is innervated
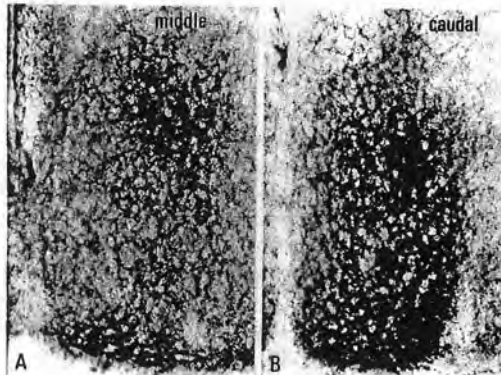

Fig. 1 Coronal section of the hamster SCN through the middle (A) and caudal (B) level showing the distribution of PACAP-immunoreactivity.

apart from the retina, also from the intergeniculate leaflet [6] and either one or both of these two sources are therefore likely origins of the PACAPergic input.

Several lines of evidence in the rat suggest that the major portion of the PACAPergic input actually originates from retinal ganglion cells [4]. Firstly, PACAP-positive ganglion cells are observed displaying a morphological type that resemble those earlier shown to project to the circadian timing system [7]. Secondly, bilateral enucleation produced a marked reduction in the content of PACAP-containing material in the SCN. Thirdly, neural tract tracing from the eye combined with immunostaining for PACAP showed that PACAP was present in nerve fibers and terminals in the SCN of retinal origin.

A great deal is known about how the RHT mediates photic input to the clock at night. Several studies have shown that this effect is mediated through a release of glutamate and subsequent NMDA receptor activation in the SCN [8,9]. It is therefore speculated that PACAP interacts with the glutamatergic neurotransmission in the SCN.
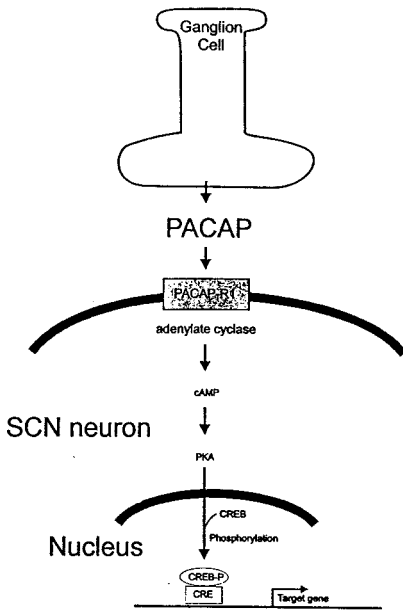

Fig. 2 Proposed signalling pathways activated by the RHT via a release of PACAP, adenylate cyclase, protein kinase A (PKA), cyclic response element binding protein (CREB). The phosphorylated form of CREB (CREB-P) binds to a CRE located on the promotor gene of yet unknown target genes.

2. PACAP-R1 receptors in the SCN

PACAP binds to specific receptors (PACAP-R) that are divided into two subtypes. VIP-R1/PACAP-R and VIP-R2/PACAP-R bind PACAP with equal affinity as the closely associated peptide, vasoactive intestinal peptide (VIP). In contrast, PACAP-R1 binds PACAP much stronger than VIP [10]. In situ hybridisation studies have revealed the presence of PACAP-R1 and VIP-R2/PACAP-R in the rat SCN [4,11]. This suggests that PACAP exerts its actions in the SCN via two distinct receptor sites, one of which is shared with VIP. Whereas PACAP-R1 mRNA is predominantly expressed in the retinorecipient area of the SCN, the VIP-R2/PACAP-R mRNA is mostly found outside this area. This suggests that PACAP, when released from retinal terminals, binds to PACAP-R1 in the SCN.

3. PACAP produces phase shifts in vitro through a cAMP dependent pathway

The presence of PACAP and its specific receptors in the SCN indicate that the peptide may exert an important role in phase shifting the clock. It has been shown that phase shifts can be elicited by incubating SCN in brain slices with cAMP and cAMP analogues [12]. cAMP produced large phase advances in the subjective day, but was without effect in the subjective night.

PACAP could also phase shift rat SCN in vitro through a cAMP dependent mechanism similar to the effect produced by cAMP analogues. This effect occurred in the subjective day, but not in the subjective night [4]. These data fit nicely together indicating that PACAP acts through an activation of a cAMP-dependent cascade.

4. Future directions

We can conclude from the present results that PACAP is present in the RHT and plays a functional role in phase shifting. However, it also raises a number of unresolved questions. In vivo, light is unable to produce phase shifts at CT5 despite that PACAP, present in the RHT, is active. PACAPs functional effects under in vivo conditions are yet to be determined, but if PACAP produces an effect at CT5, a likely functional correlate would be that PACAP is involved in integration of photic and non-photic effects on the clock. Nonphotic phase shifting is mediated by NPY, and NPY can block PACAP-induced phase shifts in vitro [13], indicating that a link between the two afferent systems occurs in the SCN. A speculative alternative is that PACAP modulates photic shifting through a postsynaptic mechanism. It is known that glutamate exerts its action through a NMDA receptor, and that this signal is mediated in the cell via intracellular Ca^{++}, phosphorylation of the CRE binding protein CREB, NO activation, and Fos expression [14]. PACAP exerts its effect via activation of adenylate cyclase, protein kinase A, and perhaps also by phosphorylation of CREB [15], that either activate gene transcription directly (Fig. 2) or modulate other signalling pathways in SCN neurons.

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