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# PACAP27 regulates ciliary function in primary cultures of rat brain ependymal cells

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**Abstract**

Ependymal cells line the brain ventricles and separate the CSF from the underlying neuronal tissue. The function of ependymal cilia is largely unclear however they are reported to be involved in the regulation of CSF homeostasis and host defence against pathogens. Here we present data that implicates a role of pituitary adenylate cyclase-activating polypeptide (PACAP) in the inhibition of ependymal ciliary function, and also that the PACAP effects are not entirely dependent on adenylyl cyclase activation. Primary ependymal cultures were treated with increasing doses of PACAP27 or adenylyl cyclase toxin (ACT), and ciliary beating was recorded using high-speed digital video imaging. Ciliary beat frequency (CBF) and amplitude were determined from the videos. Ependymal CBF and ciliary amplitude were attenuated by PACAP27 in a concentration- and time-dependent manner. The peptide antagonist PACAP6-27 blocked PACAP27-induced decreases in amplitude and CBF. Treatment with ACT caused a decrease in amplitude but had no effect on CBF, this suggests that the inhibition of CBF and amplitude seen with PACAP27 may not be completely explained by $G_s$–AC-cAMP pathway. We present here the first observational study to show that activation of PAC1 receptors with PACAP27 has an important role to play in the regulation of ependymal ciliary function.


*Keywords:* Ependymal cilia; Ciliary function; Pituitary adenylate cyclase-activating polypeptide; PACAP; PAC1 receptor

## 1. Introduction

Ependymal cells lining the ventricular cavities form a protective interface between brain parenchyma and the circulating cerebrospinal fluid (CSF). Ependymal cells bear approximately 40 motile cilia per cell which beat in the coordinated pattern at approximately 40 Hz (Del Bigio, 1995; O'Callaghan et al., 1999) that rapidly move CSF adjacent to the ventricular walls. The precise functional roles of ependymal cilia are not fully understood, however, it is known that abnormal ciliary function (Banizs et al., 2005; Mönkkönen et al., 2007; Shimizu and Koto, 1992), associated with an inherited condition known as primary ciliary dyskinesia (PCD) can result in hydrocephalus (Ibañez-Tallon et al., 2004). Ependymal cilia also have been shown to play an important role in host defence against pathogens (Hirst et al., 2003, 2000; Mohammed et al., 1999). In addition, a recent study (Sawamoto et al., 2006) has shown that ependymal cilia are responsible for the formation of gradients of CSF guidance molecules that direct neuroblast movement in the brain parenchyma. However, few studies have addressed the question of hormonal regulation of the ciliary function.

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There is previous evidence that rat ependymal ciliary function could be inhibited by an excess activity of the adenylyl cyclase (AC) – cAMP pathway (Mönkkönen et al., 2007). The dual balance between G protein families $G_i$ and $G_s$ in the regulation of AC is well established, but G protein-coupled receptors have not been identified in CBF inhibition. Interestingly, a recent study showed that mice overexpressing PAC1 receptors developed hydrocephalus with excess activity of PKA pathway (Lang et al., 2006). PAC1 receptors are activated by pituitary adenylate cyclase-activating polypeptide (PACAP) and coupled to $G_s$ protein family. This suggests that endogenous neuropeptide, PACAP, may have a receptor-dependent role in the regulation of ciliary function.

During evolution, PACAP has been remarkably well conserved, and it shares a striking sequence similarity between species (Arimura, 1998) and suggests a vital physiological role. PACAP is a pleiotropic peptide, involved in the regulation of hormone release from the pituitary and adrenal gland. PACAP has been implicated in a variety of biological processes such as reproduction, development, growth, immune responses, cardiovascular, gastrointestinal and respiratory functions (Arimura, 1998; Vaudry et al., 2000). In the CNS, PACAP acts as a neurotrophic factor during brain development and as a neuroprotective factor during adulthood (Arimura, 1998; Vaudry et al., 2000; Watanabe et al., 2007). Further, it is a neurotransmitter and/or a neuromodulator in the PNS and a preganglionic neurotransmitter in the CNS (Arimura, 1998). Physiological concentrations of PACAP in CSF and brain tissue are in the range of 0.1–2 nM (Ohno et al., 2005; Wilderman and Armstead, 1997). In rat brain, high levels of PACAP are mainly found in the hypothalamus. PACAP acts as the transmitter of the monosynaptic neuronal pathway (the retinohypothalamic tract) mediating light information to the master biological clock, the hypothalamic suprachiasmatic nuclei (Hannibal, 2006). In addition, regions adjacent to the ventricles, like septal, paraventricular and periventricular nuclei, have high PACAP concentrations as well as high density of PACAP immunopositive cells and fibers in both rat and human (Arimura et al., 1991; Ghatei et al., 1993; Köves et al., 1991; Masuo et al.;1993; Palkovits et al., 1995; Vigh et al., 1991).

We have previously described a primary rat ependymal culture model that allows ciliary function to be studied over time (Hirst et al., 2000). An often ignored fact regarding the ciliary function is that following certain topical toxic insults the CBF remains unchanged whilst the ciliary amplitude, and hence function, may be disturbed (Chilvers and O'Callaghan, 2000). The present study allowed us to use our methodology to determine the role of PACAP in both ependymal CBF and amplitude.

## 2. Materials and methods

### 2.1. Ependymal cell culture

Ciliated rat ependymal cells were grown using a method adapted from Wiebel and colleagues (Weibel et al., 1986) and described previously (Hirst et al., 2000).

### 2.2. Compound addition

All experiments were performed in Hepes-buffered minimum essential media (MEM) without additives. PACAP27 (Tocris, Bristol, UK), PACAP6-27 (Sigma–Aldrich, Poole, UK) and adenylyl cyclase toxin (Sigma–Aldrich) were diluted to their final concentrations in 1 ml MEM. Negative controls were incubated in 1 ml of MEM alone. The compounds were added on the cells at 0 min (all compounds used), 120 min (PACAP27, PACAP27 combined with PACAP6-27, ACT) and 240 min (PACAP27) in increasing concentrations. In the case of PACAP27 and peptide antagonist PACAP6-27 combination, the cells were pre-treated for 60 min with the peptide antagonist.

### 2.3. CBF and amplitude

To determine CBF and amplitude the cells were placed in a humidified (70–90%) incubation (37 °C) chamber and observed from above using an inverted microscope system (Diphot, Nikon, UK). In order to prevent cilia-to-cilia variation, we selected a long strip of mature cilia for each recording, and followed the function of the same cilia throughout the experiment. From each recording, five cilia were randomly selected for CBF and amplitude analysis. To avoid any influence of potentially varying degree of beat pattern on the results, only the mature cilia which displayed their full beat cycle when viewed from above were included. Beating cilia were recorded using a digital high-speed video camera (Troubleshooter, Lake Image Systems, UK) at a rate of 500 frames per sec using a shutter speed of one in 2000. The camera allows video sequences to be recorded and played back at reduced frame rates or frame by frame. CBF was determined by timing a given number of individual cilia beat cycles. Basal CBF was measured at 0 min, before addition of compounds. Calculation of CBF (Hz): Frequency (Hz) = Number of frames recorded per second/frames elapsed for five ciliary beat cycles × five (conversion per beat cycle). Ciliary amplitude was measured using a similar method as previously described (O'Callaghan et al., 2008). The captured video sequences were played back at a slow rate which allowed determination of the distance travelled by the cilia tips within the power stroke of the beat cycle.