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Application of long-term microdialysis in circadian rhythm research

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Abstract

Our laboratory has pioneered *long-term* microdialysis to monitor pineal melatonin secretion in living animals across multiple circadian cycles. There are numerous advantages of this approach for rhythm analysis: (1) we can precisely define melatonin onset and offset phases; (2) melatonin is a reliable and stable neuroendocrine output of the circadian clock (versus behavioral output which is sensitive to stress or other factors); (3) melatonin measurements can be performed extremely frequently, permitting high temporal resolution (10 min sampling intervals), which allows detection of slight changes in phase; (4) the measurements can be performed for more than four weeks, allowing perturbations of the circadian clock to be followed long-term in the same animals; (5) this is an automated process (microdialysis coupled with on-line HPLC analysis), which increases accuracy and bypasses the labor-intensive and error-prone manual handling of dialysis samples; and (6) our approach allows real-time investigation of circadian rhythm function and permits appropriate timely adjustments of experimental conditions. The longevity of microdialysis probes, the key to the success of this approach, depends at least in part on the methods of the construction and implantation of dialysis probes. In this article, we have detailed the procedures of construction and surgical implantation of microdialysis probes used currently in our laboratory, which are significantly improved from our previous methods.

Keywords: Circadian rhythms; Pineal; Melatonin; Microdialysis; Suprachiasmatic nucleus

1. Introduction

Circadian rhythms of behavior and physiology are controlled by a central pacemaker located in the suprachiasmatic nucleus (SCN) in mammals (Moore-Ede, 1982). Unlike many physiological events that remain more or less constant during the course of a day, rhythmic events such as hormone secretion and sleep–wake cycles display dynamic changes across the diurnal cycle. These dynamic changes can be accurately followed in vivo most effectively via continuous and high-resolution monitoring of individual animals. Because it is extremely difficult to directly investigate the pacemaker properties of the SCN in vivo, studies of the circadian pacemaker in freely moving animals have relied largely on the monitoring of locomotor activity rhythms under various experimental conditions (Aschoff et al., 1975; Pittendrigh and Daan, 1976). Studies that utilize behavior rhythms as the clock marker are beyond the scope of this chapter.

Properties of the circadian pacemaker have also been studied by monitoring in vivo secretion of circadian hormones such as melatonin using a microdialysis probe inserted into the pineal gland (Azekawa et al., 1990, 1991; Drifhgout et al., 1993; Barassin et al., 1999; Kalsbeek et al., 2000), the site of melatonin production (Borjigin et al., 1999). Melatonin production in the pineal gland is controlled by the circadian pacemaker in the SCN via a multi-synaptic neuronal pathway (Fig. 1A; Kennaway, 1997). Among the common circadian markers used in rhythm studies, including sleep–wake, activity–rest, body temperature, melatonin and cortisol secretion rhythms, melatonin is consistently recognized as the best marker of the circadian pacemaker (Lewy et al., 1999; Klerman et al., 2002; Arendt, 2005).

Melatonin is synthesized from tryptophan via four enzymatic steps (Fig. 1B; Klein et al., 1992). Tryptophan hydroxylase 1 (TPH1) catalyzes the first reaction that converts tryptophan to 5-hydroxytrypophan, which is subsequently converted to 5-hydroxytryptamine (5-HT or serotonin), by aromatic amino acid decarboxylase (AADC). Both reactions occur in vivo during the

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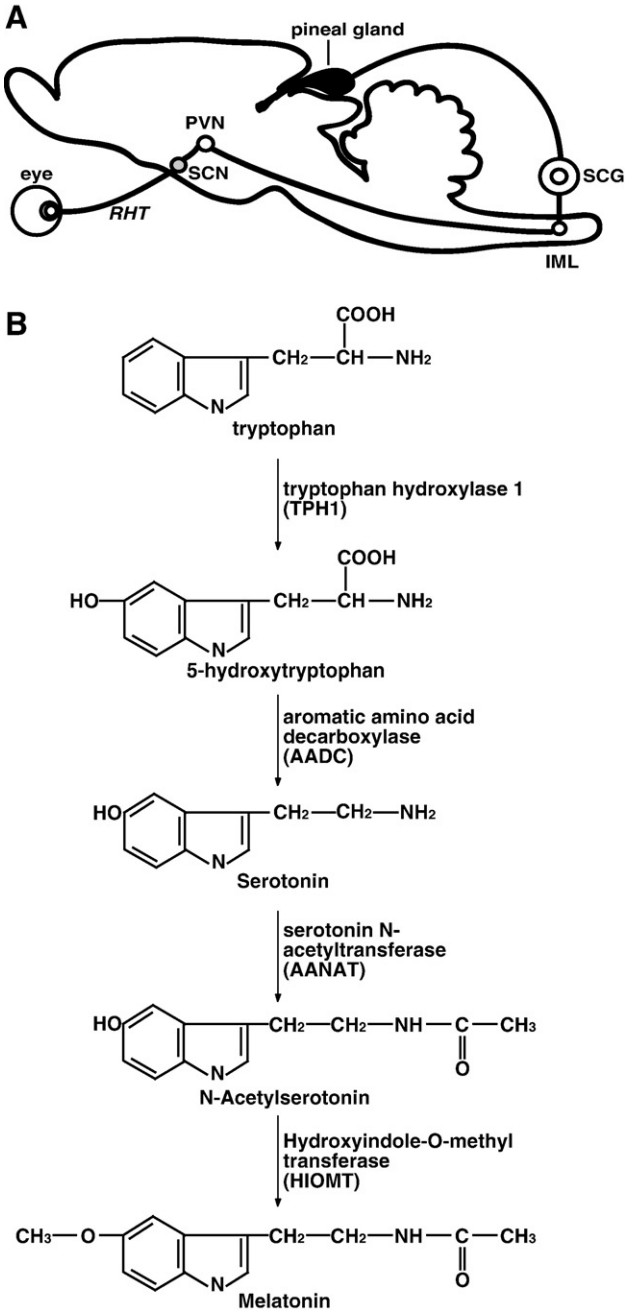

Fig. 1. Pineal melatonin synthesis. A. Melatonin production in the pineal gland is controlled by the circadian pacemaker in the SCN via a multi-synaptic pathway that includes the suprachiasmatic nucleus (SCN), the paraventricular nucleus of the hypothalamus (PVN), intermediolateral nucleus of the spinal cord (IML), and the superior cervical ganglion (SCG). Light signals from the eye, transmitted to the SCN via the retinohypothalamic pathway (RHT), entrain the circadian clock in the SCN, which generates the circadian rhythms of melatonin production in the pineal gland. B. Melatonin is synthesized from tryptophan by four enzymes: tryptophan hydroxylase 1 (TPH1), aromatic amino acid decarboxylase (AADC), arylalkylamine *N*-acetyltransferase (AANAT), and hydroxyindole-*O*-methyltransferase (HIOMT). In our studies, we routinely collect data for serotonin, *N*-acetylserotonin, and melatonin in a single HPLC chromatogram.

daytime as well as at night. The last two reactions, which are active at night, are mediated by arylalkylamine *N*-acetyltransferase (AANAT) that converts serotonin to *N*-acetylserotonin (NAS) and by hydroxyindole-*O*-methyltransferase (HIOMT) that forms melatonin (Fig. 1B).

In earlier experiments, microdialysis sampling and analyses of melatonin were carried out in vivo for 1–4 days in individual animals, and the results were averaged for a group of animals (Azekawa et al., 1990, 1991; Drifhgout et al., 1993; Kalsbeek et al., 2000). Inter-individual variations of the circadian pacemaker activity were difficult to define. We have pioneered the long-term pineal microdialysis technique that allows continuous on-line sampling and analysis of melatonin for more than 4 weeks in individual animals (Sun et al., 2002, 2003). This powerful technique enabled us to closely follow minute changes of the circadian status of individual animals for prolonged periods under various experimental conditions (Sun et al., 2003), allowed us to identify inter-individual differences in circadian chronotypes in laboratory animals (Liu and Borjigin, 2006), and permitted an in vivo investigation of the circadian pacemaker in a manner that is difficult using conventional approaches (Liu and Borjigin, 2005a,b). We believe that the success of our approach depends largely on the design and surgical implantation of the pineal microdialysis probe, which will be detailed in this chapter.

2. Experimental procedures

In our earlier publications (Sun et al., 2002, 2003; Liu and Borjigin, 2005a,b,c, 2006), a concentric probe from commercial sources was used. Unlike transverse probes that can cause tissue damage along their path into the brain (Drijfhout et al., 1993), the concentric probes inserted using our technique caused very little damage to the brain tissue surrounding the pineal gland. This unique feature of our previous method of surgical implantation was thought to be responsible for the in vivo longevity of the microdialysis probes in our studies (Sun et al., 2003). Because the pineal gland is located directly below the confluence of the superior sagittal sinus and the transverse sinus, the surgical implantation of the concentric probe was technically challenging (Sun et al., 2003). Recently, we have significantly improved the construction and surgical implantation of transverse microdialysis probes and have successfully used this approach for long-term microdialysis of the pineal. Compared to our previous method, this latest approach is far less costly and greatly simplifies the method of surgical implantation of the probe. The methods presented in this chapter are those currently used in our laboratory.

2.1. Probe construction

A transverse microdialysis probe depicted in Fig. 2 is used in our laboratory. The support portion of the probe is constructed from blunt tip needles of two different configurations. The outer support is provided by a 21-gauge blunt tip needle with a shaft length of 0.5″. This added support, one of our latest improvements in probe construction, makes the probe virtually impossible to bend during dialysis and greatly enhances the longevity of the probe. The inner tubing in direct contact with the dialysis samples is constructed from a 25-gauge blunt tip