Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John’s wort (Hypericum perforatum L. cv. Anthos) plants

Abstract Evidence of a pathway for the biosynthesis of the mammalian neurohormones melatonin and serotonin in in vitro regenerated plantlets of St. John’s wort (Hypericum perforatum cv. Anthos) is presented. Isotope tracer experiments were performed on plantlets regenerated from thidiazuron-induced stem explants and grown on MS basal medium for 2 months. Radiolabel from 14C-tryptophan was recovered as 14C-indoleacetic acid, 14C-tryptamine, 14C-5-hydroxytryptophan, 14C-serotonin and 14C-melatonin in the treated St. John’s wort plantlets. Chromatographic peak identity was confirmed by high performance liquid chromatography-mass spectrometry-mass spectrometry and quantification of melatonin by radioimmunoassay. Significantly more radiolabel was recovered in serotonin relative to melatonin under low light conditions with this ratio being reversed under increased lighting, indicating that the rate of flow through this biosynthetic pathway is regulated, at least in part, by light.

Key words Tryptophan · Indoleacetic acid · Melatonin · Serotonin · St. John’s wort

Introduction

In plant tissues, the primary metabolites of tryptophan are auxin, glucosinolates, phytoalexins, alkaloids and indoles (Radwanski and Last 1995). Of these, the most highly characterized metabolite of tryptophan is the plant hormone indoleacetic acid (IAA; Bartel 1997), which exerts a strong biological activity at very low concentrations and is essential for maintenance of physiological processes in plants in both in vitro and in vivo. Although the primary precursor for auxin biosynthesis is thought to be tryptophan, it has been difficult to unequivocally identify a single biosynthetic pathway for IAA, since there may be several precursors and conjugated forms of IAA (Bartel 1997). Also, results generated from various plant species indicate that in some species or stages of development, IAA may be produced from an alternate indole biosynthetic pathway (Michalczuk et al. 1992; Östin et al. 1999).

We previously reported significantly higher levels of melatonin, used in the treatment of neurological disorders, in several medicinal plants including St. John’s wort (Murch et al. 1997). Although there is currently no known role for melatonin in plant morphogenesis or physiology, Balzer and Hardeland (1996) hypothesized that melatonin in plants may have an analogous role to that in mammals, acting as a chemical messenger of light and dark, a calmodulin binding factor or as an antioxidant. In this way, the relative ratios of melatonin and serotonin may be involved in regulation of light:dark responses, seasonality and circadian rhythms in plants (Balzer and Hardeland 1996; Kolar et al. 1997). However, a biosynthetic pathway for serotonin and melatonin has not been established in a higher plant species. In mammals, yeast and bacteria, melatonin is synthesized from tryptophan via 5-hydroxytryptophan and serotonin (Fig. 1) and the rate of flow of metabolites through this pathway in the mammalian system is
responsive to both metabolic and environmental factors (Yu and Reiter 1993; Balzer and Hardeland 1996). The objective of this study was to investigate the potential occurrence of the melatonin biosynthetic pathway in St. John’s wort and to quantify the potential incorporation of radiolabel from tryptophan into auxin and indoleamine metabolites under low and supplemental light conditions.

**Materials and methods**

**In vitro culture of St. John’s wort**

Seeds of St. John’s wort (*Hypericum perforatum* L. cv Anthos) were germinated as described previously (Murch et al. in press). Nine stem cuttings, approximately 1 cm long, were cultured on a medium containing MS salts (Murashige and Skoog 1962) with B5 vitamins (Gamborg et al. 1968), 30 g l⁻¹ sucrose (hereafter referred to as MSO), supplemented with varying levels (0, 5, 10, 15 and 20 μmol l⁻¹) of the growth regulator thidiazuron (TDZ; N-phenyl-N’-(1,2,3-thiadiazol-yl)urea; Sigma, St. Louis, Mo.). The pH was adjusted to 5.7 and 3 g l⁻¹ gellan gum (Gelrite, Schweitzerhall, South Plainfield, N.J.) was added before the medium was autoclaved. The optimal time of exposure to TDZ was determined in explant cultures transferred onto MS after 3, 6, 9 and 12 days of incubation. All cultures were incubated in a growth cabinet with a 16 h photoperiod under cool white light at 40–60 μmol m⁻² s⁻¹ (model F40/CW/RS/EW-II Philips, Scarborough, Ontario, Canada). The effect of TDZ on regeneration of stem cuttings was quantified after 18 and 23 days of culture.

**Statistical analyses**

The design for all regeneration experiments was a complete randomized block and all treatments consisted of five replications. All the experiments were repeated at least twice and the data were analysed using SAS Version 6.12 (SAS 1995). Significant differences between means were assessed by a Student-Neuman-Keuls means separation test at $P \leq 0.05$.

**14C-tryptophan metabolism**

Regenerated plantlets were subcultured in Magenta boxes containing 50 ml of MSO 2 months prior to radioisotope tracer studies. To ensure that there was no residual bacterial or fungal population in the cultured plantlets, four plantlets were homogenized in 1.0 ml of phosphate buffered saline and the resulting slurry was incubated on nutrient agar (Bacto-Difco) at 30°C for 7 days.

Individual plantlets, about 15 cm long, were separated from the culture boxes and acclimatized to experimental conditions in the culture vessels for 3 h prior to infusion. Plantlets were transferred to Eppendorf tubes containing 200 μl of the 14C-tryptophan infusate solution (370 kBq l⁻¹; 14C-(3-sidechain)-tryptophan, Dupont/New England Nuclear, Mass.) in half-strength liquid MSO. Over the 60-min infusion period, samples were collected at 10-min intervals, immediately immersed in liquid nitrogen and stored at −80°C until analysis. The relative rate of incorporation of label was determined with 14C-radioisotope infusion studies under low light (6 μmol m⁻² s⁻¹) and supplemental light (40 μmol m⁻² s⁻¹) conditions provided by cool white fluorescent tubes.

**Sample preparation**

Frozen samples were ground in 100 μl of Tris buffer (1 M Tris-HCl, pH 8.4) and prepared as described by Poeggeler and Hard-