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Changes in Thyroid Hormone Concentrations after Administration of Ashwagandha Root Extract to Adult Male Mice

SUNANDA PANDA AND ANAND KAR

School of Life Sciences, D. A. University, Vigyan Bhavan, Khandwa Road, Indore-452 017, India

Abstract

The importance of ashwagandha root extract in the regulation of thyroid function with special reference to type-I iodothyronine 5′-monodeiodinase activity in mice liver has been investigated.

Although the root extract ($1{\cdot}4\,g\,kg^{-1}$) administered daily for 20 days by gastric intubation increased serum 3,3′,5-triiodothyronine (T3) and tetraiodothyronine (T4) concentrations and hepatic glucose-6-phosphatase activity, hepatic iodothyronine 5′-monodeiodinase activity did not change significantly. Furthermore, ashwagandha root extract significantly reduced hepatic lipid peroxidation, whereas the activity of antioxidant enzymes such as superoxide dismutase and catalase were increased.

These findings reveal that the ashwagandha root extract stimulates thyroidal activity and also enhances the antiperoxidation of hepatic tissue.

Ashwagandha (*Withania somnifera*, family Solanaceae), a subtropical undershrub is highly esteemed as a potent herb in Ayurveda (Indian medicinal system). Of all the parts of the plant the root has been reported to be pharmacologically most active and is predominantly used for therapeutic purposes (Tripathy et al 1996). Some of the medicinal properties attributed to this plant are sedative, hypotensive, anti-ageing, aphrodisiac, anti-inflammatory, bradycardiac, respiration stimulating, anti-tumour and radiosensitizing (Malhotra et al 1960a, b; Uma Devi 1996). In Indian traditional medicine ashwagandha root extract is used as a health tonic and is also prescribed for some common diseases of the reproductive tract, for gastrointestinal disorders and for glandular swellings (Tripathy et al 1996). In recent investigations we have reported the protective free-radical-scavenging role of ashwagandha root extract in mouse liver (Panda & Kar 1997c, Panda et al 1997), the organ where most circulatory triiodothyronine (T3) is generated (Visser et al 1978).

Although ashwagandha is one of the most investigated medicinal plants, there have been no reports about its efficacy in the regulation of thyroid function, except a preliminary report on the cockerel (Panda & Kar 1997a), indeed there are few reports on thyroidal regulation by plant extracts (Dhawan & Goel 1994; Winterhoff et al 1994; Tripathy & Chaturvedi 1995; Panda & Kar 1997b).

This paper reports a study of the regulation of thyroid function in the male mouse with ashwagandha root extract. An attempt has also been made to correlate changes in thyroid hormone concentrations with changes in the peroxidative process in liver tissue.

Correspondence: A. Kar, School of Life Sciences, D. A. University, Vigyan Bhavan, Khandwa Road, Indore-452 017, India.

Materials and Methods

Aqueous plant extract was prepared in a Soxhlet apparatus from dried root powder of ashwagandha (containing 1·75% withanolides), supplied by Kisalaya Pharmaceuticals (Indore, India).

Twenty adult healthy colony-bred Swiss albino male mice, 28 ± 2 g were maintained under conditions of constant temperature (27 ± 1°C) and light (14 h light and 10 h dark) with food and water freely available. They were divided into two groups of ten and the initial body weight of each was recorded. Group I received vehicle (distilled water) and served as control; group II was treated with $1{\cdot}4\,g\,kg^{-1}\,day^{-1}$ (Panda & Kar 1997c) ashwagandha root extract daily for 20 days by gastric

intubation. The plant extract was always administered between 1100 and 1130h to avoid circadian interference. On the last day, animals were killed after measurement of the final body weight.

After exsanguination, the liver was removed quickly, washed thoroughly with phosphate-buffered saline (PBS, pH 7·4) and processed for biochemical estimations. Lipid peroxidation was determined by reaction of thiobarbituric acid with malondialdehyde, formed by peroxidation of lipids, by the method of Ohkawa et al (1979). Superoxide dismutase activity was estimated by measuring the percentage inhibition by the enzyme of the autoxidation of pyrogallol, according to the method of Marklund & Marklund (1974). One unit of superoxide dismutase was defined as the enzyme activity that inhibits the autoxidation of pyrogallol by 50%. Catalase activity was estimated by the method of Aebi (1983) and was expressed as μmol H_2O_2 decomposed min^{-1} (mg protein)$^{-1}$. Glucose-6-phosphatase activity was measured by the method of Baginske et al (1974) and hepatic protein content by the method of Lowry et al (1951).

Serum concentrations of total T3 and T4 were measured by radioimmunoassay as reported elsewhere (Maiti & Kar 1997; Panda & Kar 1997a, b) using the protocol and radioimmunoassay kits of the Bhabha Atomic Research Centre, Bombay, India. Measurement of iodothyronine 5′-monodeiodinase was performed by specific radioimmunoassay for determination of in-vitro generation of T3 in the ethanolic extract of hepatic tissue by the method of Kahl et al (1984).

Data are expressed as means ± s.e.m. Statistical evaluation of the data was performed by Students *t*-test for the difference of means; statistical significance was ascribed at $P < 0.05$.

Table 1. Effects of ashwagandha root extract (1·4 g kg^{-1}) daily for 20 days on serum T3 and T4 concentrations and on hepatic iodothyronine 5′-monodeiodinase activity in the male mouse.

	Control	Treated
3,3′,5-Triiodothyronine (ng mL^{-1})	2·2 ± 0·1	2·6 ± 0·1*
Tetraiodothyronine (ng mL^{-1})	52·2 ± 11·5	110 ± 6·2**
Iodothyronine 5′-monodeiodinase (ng T3 h^{-1} (mg protein)$^{-1}$ × 10^{-1})	1·2 ± 3·7	4·4 ± 0·5

Data are means ± s.e.m. (n = 10). *$P < 0.05$, **$P < 0.001$, significantly different from respective control values.

Results

Results are summarized in Tables 1 and 2. Although there was no significant difference between the initial and final body weights of the animals (data not shown), in both groups serum T4 and T3 concentrations increased significantly ($P < 0.001$ and $P < 0.05$ respectively when compared with their respective control values; Table 1) after treatment with ashwagandha. However, iodothyronine 5′-monodeiodinase activity did not change significantly. Significant increases in hepatic glucose-6-phosphatase ($P < 0.01$), superoxide dismutase ($P < 0.001$) and catalase ($P < 0.01$) activity was observed in the treated animals (Table 2); lipid peroxidation decreased significantly ($P < 0.01$) after drug administration. All the animals were healthy and active throughout the experiment.

Discussion

Our results clearly indicate that ashwagandha root extract enhances the levels of circulating thyroid hormones as evidenced by an increase in serum T4 and T3 concentrations. Other plants have been found to have similar prothyroidic effects (Dhawan & Goel 1994; Tripathy & Chaturvedi 1995) and we also observed the prothyroidic action of ashwagandha root extract in an earlier study in the cockerel (Panda & Kar 1997a).

Table 2. Effects of ashwagandha root extract (1·4 g^{-1} kg^{-1}) daily for 20 days on hepatic lipid peroxidation, superoxide dismutase, catalase and glucose-6-phosphatase activity in the mouse.

Group	Lipid peroxidation (nmol malondialdehyde h^{-1} (mg protein)$^{-1}$)	Superoxide dismutase (units (mg protein)$^{-1}$)	Catalase (μmol H_2O_2 decomposed h^{-1} (mg protein)$^{-1}$)	Glucose-6-phosphatase (μmol phosphate h^{-1} (mg protein)$^{-1}$)
Control	0·409 ± 0·022	5·55 ± 0·17	53·15 ± 1·92	0·007 ± 0·001
Treated	0·208 ± 0·046**	8·29 ± 0·25***	63·86 ± 3·079**	0·015 ± 0·002**

Data are means ± s.e.m. (n = 10). **$P < 0.01$, ***$P < 0.001$, significantly different from respective control values.

Treatment with the drug resulted in a substantial increase (approx. 111%) in the level of T4 in serum; the increase in the level of T3 was less (only 18%). Because T4 is the predominant circulating thyroid hormone and is synthesized solely in the thyroid gland (Greenspan 1994) the increase in T4 concentration in the drug-treated animals suggests that the plant extract primarily stimulates thyroid gland to synthesize or secrete (or both) thyroxine. Although the thyroid also synthesizes and secretes T3, the gland accounts for only 13% of the total circulating level, the major amount of this hormone being derived by peripheral monodeiodination of T4 (Visser et al 1978; Kuhn et al 1988; Ganong 1995) by the enzyme iodothyronine 5′-monodeiodinase. This investigation observed no significant change in monodeiodinase activity in the drug-treated animals, suggesting that the plant extract has little or no role in the regulation of extrathyroidal conversion of T4 to T3 and so the small but significant increase in serum T3 concentration could also be a result of drug-induced stimulation of intrathyroidal hormone synthesis rather than extrathyroidal conversion of T4 to T3.

Carbohydrate metabolism is also influenced by thyroid hormones. In this experiment the plant extract was found to increase the activity in the liver of a key enzyme of thyroid function, glucose-6-phosphatase, coincident with increased thyroid hormone concentrations. This could be a secondary effect of the drug, because exogenous T4 is known to enhance the activity of this enzyme (Muller et al 1988).

In recent years lipid peroxidation has been linked with damage to tissue membranes and has been shown to be involved in variety of pathological phenomena (Cand & Verdetti 1989). In the drug-treated animals hepatic lipid peroxidation was inhibited, indicating its antiperoxidative role in liver tissue. This was further supported by an increase in the activity of superoxide dismutase and of catalase, known to be endogenous antioxidative enzymes, possibly as a result of the protein anabolic nature of the thyroid hormones (Turner & Bagnara 1976) or, as described earlier, as a result of a direct drug-induced increase in serum T4 concentration (Pereira et al 1995), further supporting the prothyroidic role of the plant extract.

These observations suggest that at the dose used the drug is not hepatotoxic but rather it reduces the peroxidation of membrane lipids in liver tissue, which accords with our earlier reports (Bhattachary et al 1997; Panda et al 1997; Panda & Kar 1997b).

In summary, ashwagandha root extract might act both as a prothyroidic agent and as an antiperoxidative agent. However, for optimization of the dose required and the duration of treatment of hypothyroidism, further investigation is required.

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