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Hypothermic effects of hops are antagonized with the competitive melatonin receptor antagonist luzindole in mice

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Abstract

Hops (*Humulus lupulus*, Cannabinaceae) has been used in traditional European medicine as a mild sedative for the treatment of anxiety, nervousness, and insomnia. However, there has been little information available about the underlying sleep inducing mechanism of hops. We have investigated the effects of a hops extract on the rectal body temperature in mice. Hops extract (250 mg kg^{-1}) significantly decreased body temperature in male BL6/C57J mice ($\triangle$T -0.75 ± 0.07°C) 2 h after oral administration. The effects of the plant extract were comparable with melatonin (50 mg kg^{-1}; $\triangle$T -0.66 ± 0.06°C; 2 h after i.p. injection). The hypothermic effects of melatonin and hops extract were antagonized with the competitive melatonin receptor antagonist luzindole. Thus, our data suggests that the hypothermic—and therefore the sleep-inducing—effects of hops extract are possibly mediated through activation of melatonin receptors.

Introduction

Humulus lupulus L. is a member of the hemp family (Cannabinaceae), which is cultivated in temperate regions of the world. The female flowers mature in summer and are used to add bitterness, flavour and aroma to beer. In ancient times the young shoots were eaten as a vegetable, and the dried flowers were used for their slight narcotic effect and sedative action in the treatment of mania, toothache, earache and neuralgia (Haas 1995). In modern phytotherapy hops is used as a sedative and mild hypnotic (Haensel et al 1982; Lee et al 1993), as well as for its estrogenic (Goetz 1990; Milligan et al 1999), free radical scavenging (Tagashira et al 1995) and antitumour properties (Miranda et al 1999; Shimamura et al 2001; Chen & Lin 2004). Much of the use of hops as a mild sedative came from the observation of sleepiness in hop-pickers (Schulz & Haensel 2004). The Complete German Commission E Monographs list hops as an approved herb for "mood disturbances such as restlessness and anxiety, sleep disturbances" (Blumenthal 1998). The number of clinical studies supporting the use of hops as a sedative is still limited; however, several European studies have demonstrated formulas combining hops with other sedative drugs (such as valerian root or passion flower) as being effective for the treatment of sleeplessness (Schmitz & Jackel 1998; Fussel et al 2000; Vonderheid-Guth et al 2000; Morin et al 2005). In addition, a few pharmacological studies have investigated the central actions of hops. In an animal study, Bravo et al (1974) demonstrated a tranquilizing effect in mice. Lee et al (1993) evaluated the CNS effects of a hops extract in mice and reported hypothermic, analgesic, anticonvulsant and hypnotic properties after intraperitoneal administration. Hypothermic and hypnotic effects were also reported after oral administration of different hops extracts (Schiller 2002). Zanoli et al (2005) showed that a lipophilic hops extract prepared by CO_2-extraction and its fractions exerted antidepressant-like activity in the forced swimming test in rats. Abourashed et al (2004) have shown that hops extract had considerable affinity towards the serotonin (5-HT_{4e}, 5-HT_6 and 5-HT_7) and melatonin (ML_1 and ML_2) receptors.

Melatonin is known to have hypnotic and hypothermic effects at physiological levels. Indeed, the hypnotic effect may be mediated via the hypothermic action (Gilbert et al 1999).

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The above considerations and the traditional use of hops as a sleep inducer prompted us to evaluate the hypothermic activity of hops extract in mice.

Materials and Methods

Animals

Male Black Six mice (21–30 g; C57BL/6J, Harlan, Indianapolis, IN) were used in this study. Animals were housed in groups of five at 20 ± 1°C with a 12-h light/dark cycle. Food (Teklat LM-485, Harlan Teklad, Indianapolis, IN) and water were freely available. Experiments were carried out between 0900 and 1300 h. A total of n = 10 animals per treatment group were used for the experiments. The sample size for each treatment group was determined by PS Power & Sample Size Calculation (version 2.1.31, Plummer, D., Vanderbilt Medical Center, Nashville, TN) for paired studies with 90% power, 95% confidence interval, expected standard deviation of 20 and expected difference of 20. The result was 10 mice per treatment group. All animals were housed and all experiments performed according to the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville (NIH publication #85-23).

Chemicals and hops extract

Melatonin was purchased from Sigma-Aldrich Co. (St Louis, MO). Luzindole was purchased from Toronto Research Chemicals Inc. (North York, ON). The hops extract was kindly contributed from Zeller AG (Romanshorn, Switzerland). The extract was prepared from the dried cones of *H. lupulus* L. (Humuli lupuli strobuli Ph. Eur.) by maceration with methanol/water 45% (w/w) at ambient temperature for 2 h with stirring. The extract was concentrated under vacuum and spray-dried with the addition of 30% maltodextrin (Ph. Eur. quality), relative to the total dry mass. The drug-extract ratio (DER) was 5–7:1 for the hop extract. Qualitative and quantitative phytochemical analysis of the extract was performed by HPLC (column: Hypersil 120-5 ODS, 5 μm, 100 × 4.6 mm; detection wave length 354 nm; solvent A, methanol; solvent B, 0.5% phosphoric acid in water; gradient: 0–8 min 33% A, 8.5–12.5 min 100% A, 13–16 min 33% A). The hops extract was standardized on an amount of 0.48% flavonoids calculated as rutin and it contained no hops bitter acids due to the hydrophilic solvent in the extraction process.

Animal experiments

For the animal experiments, hops extract was homogenously suspended in deionized water (Millipore quality) containing 1% ethanol (Aaper Alcohol and Chemical Co. Shelbyville, KY). Corresponding control animals received 1% ethanol solution. Both compounds were administered orally by means of a feeding needle one hour before the measurement of rectal body temperature. Melatonin was dissolved in ethanol and was then further diluted with isotonic saline to a final ethanol concentration of 5%. Corresponding control animals received 5% ethanol solution. Both treatments were given by intraperitoneal injection. The competitive melatonin antagonist luzindole was dissolved in ethanol and was then further diluted with deionized water to a final ethanol concentration of 7.5%. Luzindole (30 mg kg^{-1}) was injected intraperitoneally 15 min before administration of melatonin, hops extract, or control. The administration volume of all compounds was 10 mL kg^{-1}. Digital recordings of the temperature were determined with an accuracy of 0.01°C by means of a digital thermometer (Thermalert TH-5, Physitemp, Clifton, NJ). The probe (RET-3, Physitemp, Clifton, NJ), dipped in vegetable oil before insertion, was held into the rectum until a stable rectal temperature was measured for 10 s. Body temperature was measured at the time of administration (basal temperature), and then measurements were made every 60 min during a 3-h period.

Statistics

All statistical procedures were performed by use of the GraphPad Prism statistical software package, version 4.00 (GraphPad Software Inc., San Diego, CA). Data analysis was performed by analysis of variance with the Tukey test for multiple comparisons. Data were expressed as means ± s.e.m. Statistical significance was set at $P < 0.05$.

Results and Discussion

The effect on rectal body temperature in male C57BL/6J mice treated with melatonin is shown in Figure 1. Intraperitoneal administration of 25 mg kg^{-1} melatonin slightly decreased the body temperature after 120 min, but the effect was not significant when compared with the control. At a concentration of 50 mg kg^{-1} melatonin significantly lowered the body temperature 60 and 120 min after intraperitoneal administration (–0.58 ± 0.1 and –0.66 ± 0.06°C, respectively). The vehicle, 5% ethanol in saline, did not significantly alter body temperature. It took 180 min for body temperature to return to the baseline level after intraperitoneal injection of melatonin 50 mg kg^{-1}. As shown in Figure 2, hops extract at a concentration of 250 mg kg^{-1} significantly lowered body temperature

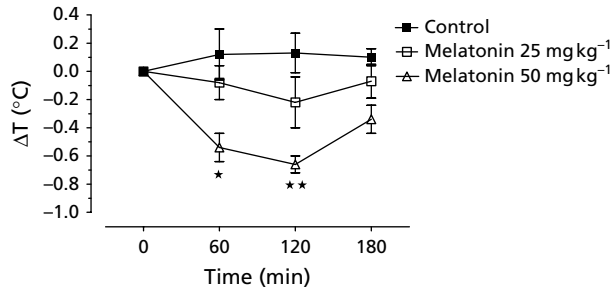

Figure 1 Effect of melatonin after intraperitoneal administration on body temperature (°C) in male C57BL/6J mice. Values are expressed as mean ± s.e.m. of n = 10 mice per group. Data were analysed by the one-way analysis of variance following Tukey's Multiple Comparison Test; *$P < 0.05$; **$P < 0.01$.

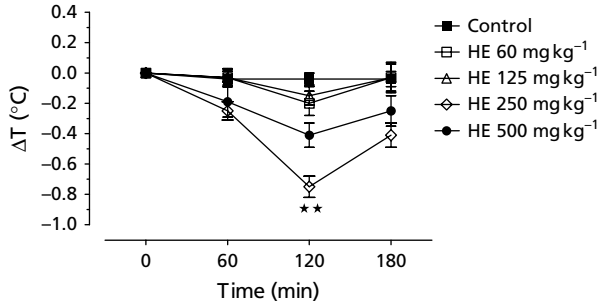

Figure 2 Effect of hops extract (HE) after oral administration on body temperature (°C) in male C57BL/6J mice. Values are expressed as mean ± s.e.m. of n = 10 mice per group. Data were analysed by the one-way analysis of variance following Tukey's Multiple Comparison Test; $^{**}P < 0.01$.

2 h after oral administration. The maximum reduction in body temperature was −0.75 ± 0.07°C. At concentrations below this (60 and 125 mg kg^{-1}, respectively) and at a higher dosage (500 mg kg^{-1}) the extract had no significant effect on body temperature, indicating a U-shaped activity. The occurrence of a U-shaped dose–response is a widely and independently observed phenomenon (Calabrese & Baldwin 2001). It has been discussed that the widespread occurrence of these U-shaped dose–responses might be examples of biological optimization processes (Calabrese et al 1999; Calabrese & Baldwin 2001).

The effects of the extract were comparable with melatonin, although the onset of action was earlier for the latter one (60 min after i.p. administration). The hypothermic effects of hops extract (250 mg kg^{-1}, p.o.) and melatonin (50 mg kg^{-1}, i.p.) were antagonized with the competitive melatonin antagonist luzindole, which was used at a concentration of 30 mg kg^{-1} (i.p. administration 15 min before drug treatment (Table 1).

The first major finding of this study was that similar to melatonin, hops extract 250 mg kg^{-1} decreased body temperature after oral administration. The second major finding was that the effects of melatonin and hops extract could be antagonized by the competitive melatonin antagonist luzindole (Dubocovich 1988).

Table 1 Effect of the competitive melatonin antagonist luzindole on body temperature (°C) in male C57BL/6J mice treated with melatonin or hops extract, respectively. Luzindole was administered intraperitoneally 15 min before administration of melatonin or hops extract

	0 min	120 min
Luzindole 30 mg kg^{-1}	36.36 ± 0.15	36.33 ± 0.11
Melatonin (50 mg kg^{-1}) + luzindole (30 mg kg^{-1})	36.35 ± 0.15	36.34 ± 0.08
Hops extract (250 mg kg^{-1}) + luzindole (30 mg kg^{-1})	36.18 ± 0.10	36.10 ± 0.09

Values were expressed as mean ± s.e.m. of n = 10 mice per group. Data were analysed by the one-way analysis of variance following Tukey's Multiple Comparison Test.

The hypothermic effects of melatonin have been examined widely (Cagnacci et al 1992; Deacon et al 1994; Hughes & Badia 1997; Van Den Heuvel et al 1999; Satoh & Mishima 2001). Reductions in core temperature can range from 0.01 to 0.4°C (Cagnacci et al 1994). Our data were in line with the observation that exogenous melatonin administration had a clear hypothermic effect. However, the dose of melatonin producing hypothermic effects varies highly in the literature. It can range between 0.1 mg (Dawson et al 1996) and 100 mg (Cagnacci et al 1995). One reason for this broad dosage range might be that melatonin as a pharmacological tool and therapeutic entity is less than ideal due to several major handicaps: the short biological half-life (approximately 19 min in rats) (Yeleswaram et al 1997), contractile effects on vascular smooth muscle (Viswanathan et al 1990), low aqueous solubility, and poor oral bioavailability. In this study we used ethanol to dissolve melatonin and diluted it further with isotonic saline. The final ethanol amount of the received melatonin suspension was 5%. Intraperitoneal administration was chosen because of the reasons mentioned above. Irrespective of its effects on the thermoregulatory systems of animals and man, melatonin has been found to have sleep-promoting properties (Dijk & Cajochen 1997; Stone et al 2000; Krauchi & Wirz-Justice 2001). The sedative and mild hypnotic properties of melatonin, and its availability as a "natural supplement", have led to widespread use of the agent for insomnia, especially in the United States. However, both uses are supported by only limited clinical trial data (Turek & Gillette 2004). Several analogues of melatonin have been developed with improved properties in comparison with melatonin (Turek & Gillette 2004).

The hypothermic effects of hops have been reported earlier (Lee et al 1993; Schiller 2002). To our knowledge, this is the first report that the hypothermic effect of hops extract was antagonized by a melatonin antagonist. It has been shown that the hops component of a valerian/hops combination (Ze 91019) showed considerable affinity towards the ML_1 receptor with an IC50 of 71 μg mL^{-1} (Abourashed et al 2004). Although hops extract was the minor component of the combination, the IC50 of the valerian/hops combination (Ze 91019) was 97 μg mL^{-1}, suggesting a synergistic interaction between valerian and hops at the ML_1 receptor (Abourashed et al 2004). Based on our data it might have been possible that the hypothermic, and therefore the sleep-inducing effects of hops extract were mediated through activation of melatonin receptors. Interestingly, the sleep-inducing effects of melatonin are reportedly correlated with its hypothermic property, suggesting that the hypnotic effect may be mediated via the hypothermic action (Gilbert et al 1999).

Conclusion

These findings have provided a new direction towards identifying new possible mechanisms of action for hops. The results seem to further exclude that hops α-acids could be responsible for the temperature lowering effects, since they were not present in the hydrophilic extract used for these experiments. Further studies are necessary to identify the active compounds in hops and to gain further insight into their precise mechanism of action.

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