Effects of St. John's Wort Extract and Single Constituents on Stress-Induced Hyperthermia in Mice

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Abstract

Emotional or stress-induced hyperthermia (SIH) is the rise of body temperature following exposure to psychological stress and has been demonstrated across species. In the present experiments we used exposure to an open field (OF) as inescapable stressor. Exposure of male BL6/C57J mice to OF stress significantly increased body temperature (ΔT = 1.8 ± 0.13 °C, $p < 0.05$). SIH is calculated as the difference ($\Delta T = T_2 - T_1$) between the basal temperature (T_1) and the temperature after exposure to an OF for 10 min (T_2). Using this experimental design, St. John's wort extract (SJW) as well as various single compounds of it were tested for their ability to affect ΔT. Anxiolytic drugs (the benzodiazepine diazepam; 5 mg/kg, and the $5HT_{1A}$ receptor agonist buspirone; 10 mg/kg) significantly reduced ΔT, whereas antidepressants (imipramine and fluoxetine) had no effect on ΔT. Oral administration of SJW extract significantly reduced ΔT in doses of 250 and 500 mg/kg. Higher (750 and 1000 mg/kg) as well as a lower dose (125 mg/kg) did not affect ΔT after stress, indicating a U-shaped dose-response curve. Hypericin (0.1 mg/kg, *p.o.*) administered 60 min prior to testing significantly decreased ΔT ($p < 0.05$) whereas hyperforin (1 – 10 mg/kg, *p.o.*) had no effect in this test paradigm. The flavonoids hyperoside, isoquercitrin and quercitrin (all at 0.6 mg/kg, *p.o.*) and rutin (1 mg/kg, *p.o.*) only partially blocked OF-induced hyperthermia. If compared to all other flavonoids, the quercetin 3-*O*-glucuronide miquelianin (1.2 mg/kg, *p.o.*) was the most potent compound tested in this experimental design. From the biflavonoids in SJW, only amentoflavone decreased SIH-induced hyperthermia in a dosage of 0.1 mg/kg. In conclusion, using open field stress as a psychological stressor to induce hyperthermia in mice we were able to detect putative anxiolytic effects of SJW extract and single consituents.

Key words

St. John's wort · hyperforin · hypericin · flavonoids · stress-induced hyperthermia · anxiolytic

Abbreviations

ANOVA:	analysis of variance
GABA:	γ-amino-butyric acid
5-HT:	5-hydroxytryptamine
OF:	open field
SIH:	stress-induced hyperthermia
SJW:	St. John's wort

Introduction

Stress plays a major role in various pathophysiological processes associated with neurodegenerative diseases and mental disorders [1]. In humans, anxiety disorders are often accompanied by an overactive autonomic nervous system, reflected in increased body temperature and heart rate [2]. Emotional or stress-induced hyperthermia (SIH) is the rise of body temperature follow-

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Received July 21, 2006 · **Accepted** September 6, 2006

Bibliography
Planta Med 2006; 72: 1366–1371 © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2006-951710 · Published online October 18, 2006
ISSN 0032-0943

ing exposure to psychological stress and has been demonstrated across species [3], [4]. In rodents, mild psychological stressors inducing hyperthermia include placing animals in novel environments (e.g., open field), restricting an animal's activity, noise, and handling animals in a variety of ways. The SIH test in mice is a paradigm developed several years ago to model the expression of autonomic hyperactivity in anxiety [5], [6]. In the SIH paradigm, group-housed mice (e.g., 10–15 mice per cage) are sequentially removed at 1-min intervals, and rectal temperatures are measured immediately upon removal [3]. While the first three mice show little change in temperature, by the last animal in the cage there is a robust increase in temperature. Also, a singly housed mouse version of this stress paradigm has been established with repeated disturbance as stressor, offering an advantage when the number of mice is the limiting factor [7]. Modifications of this method using the tail suspension test [8] or the open field [9], [10] as stressors have been published. The SIH paradigm in mice has been validated pharmacologically and has been interpreted to measure potential anti-stress and anxiolytic effects of drugs.

St. John's wort (*Hypericum perforatum* L., Clusiaceae; SJW) has been used as a medicinal herb since ancient times. Today, SJW preparations are available as an over the counter medicine in many countries for the treatment of mild to moderate forms of depression. Several clinical trials have demonstrated mood enhancement with efficacy that is comparable to widely prescribed synthetic antidepressants such as fluoxetine [11], [12], sertraline [13] and imipramine [14]. From a phytochemical point of view, SJW belongs to one of the best-investigated medicinal plants. A series of bioactive compounds has been detected in the crude material, namely phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols, some amino acids, naphthodianthrones, and essential oil constituents (for review see [15]). Although SJW has been subjected to extensive scientific studies in the last decade, there are still many open questions about the pharmacology and the mechanism of action.

A recent clinical study and three case reports have shown that SJW also exerts anxiolytic effects [16], [17]. Pharmacological studies in animals support the observations of anxiolytic and anti-stress activities of SJW [18], [19]. Since there is a strong connection between stress and selected neurodegenerative as well as mental disorders such as depression and anxiety [1], it was the aim of the present study to investigate the effect of SJW extract and several single compounds in the stress-induced hyperthermia model in mice.

Materials and Methods

Animals

Male black six mice (C57BL/6J; Harlan; Indianapolis, IN, U.S.A), housed in groups of 5 animals at 20 ± 1 °C with a 12 h light/dark cycle, receiving food (Teklat LM-485; Harlan Teklad; Indianapolis, IN, U.S.A.) and water ad libitum, and with a body weight of 21–30 g were used in the study. Experiments were carried out between 8 am and 1 pm. A total of $n = 8$ animals per treatment group was used for the experiments. All animals were adapted to the rectal temperature measurement to avoid handling stress at least one week before test begin. 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, USA (NIH publication #85–23).

Drugs

Buspirone HCl (10 mg/kg) and imipramine HCl (20 mg/kg) were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A; purity of both > 95%). Diazepam ampoules (10 mg/2 mL; Hoffmann-La Roche; Basel, Switzerland) were diluted to 5 mg/10 mL with deionized water (Millipore quality) containing 0.5% propylene glycol (Sigma-Aldrich). Fluoxetine solution (20 mg/5 mL) was purchased from Par Pharmaceutical Inc. (Spring Valley, NJ, U.S.A). The dosages of all positive controls were chosen according to the concentrations used by Olivier and co-workers [20]. St. John's wort extract (STW3 VI, DER = 3–6:1, extraction solvent: 80% ethanol; standardized on 0.3% hypericin, 4% hyperforin and 9.4% flavonoids) was provided by Steigerwald Arzneimittel GmbH (Darmstadt, Germany). Isoquercitrin (quercetin 3-*O*-glucoside; purity 95.6%) and hyperoside (quercetin 3-*O*-galactoside; purity 96.6%) were kindly provided by Tokiwa Phytochemial Co. Ltd. (Chiba, Japan). Quercitrin (quercetin 3-*O*-rhamnoside), rutin (quercetin 3-*O*-rutinoside), and amentoflavone (I3′,II8-biapigenin) (purity of all > 95%) were purchased from Chromadex (Santa Ana, CA, U.S.A). Biapigenin (I3,II8-biapigenin) (purity 99.12%), hypericin (purity 98.5%) and hyperforin (purity 93.8%) were purchased from Phytolab GmbH & Co. KG (Vestenbergsreuth, Germany). Miquelianine (quercetin 3-*O*-glucuronide, analytical data are published in [21]) was a kind gift from A. Nahrstedt (Muenster, Germany). All test compounds were dissolved in deionized water (Millipore quality) containing 0.5% propylene glycol. Since hypericin is barely soluble in water, 5 mg hypericin were dissolved in 2.5 mL ethanol and further diluted with vehicle to a final concentration of 0.1 mg or 0.5 mg per 10 mL. The final ethanol concentration of the hypericin solution was 1%. Control animals received vehicle (deionized water containing 0.5% propylene glycol) only. All substances and extracts were administered orally by a feeding needle 60 min before open field stress. The concentrations of SJW extract and single compounds used for the present study were calculated according to our previously published data [22].

Stress paradigm

One method of inducing psychological stress in rodents is exposing them to an open field (33 cm × 45 cm × 30 cm) [10]. The open field (OF) was illuminated by three 60 W lights suspended from above. The temperature in the open field box (20 ± 1 °C) was similar to that in the animals' home cage. The animals were placed into the centre of the open field box and after a period of 10 min returned to their home cage. Digital recordings of the temperature were determined with an accuracy of 0.1 °C by means of a digital thermometer (Thermalert TH-5; Physitemp; Clifton, NJ, U.S.A.). The probe (RET-3; Physitemp), dipped into vegetable oil before insertion, was held in the rectum until a stable rectal temperature was measured for 20 s. Basal temperature was recorded before the oral treatment (T_1), immediately following each other, and after the 10 min OF stress session (T_2). The open field box was cleaned with a detergent solution after each stress session to eliminate possible odor clues left by previous subjects.

Data analysis

For each individual mouse, a basal temperature (T_1), an end temperature (T_2) and the difference (ΔT) = $T_2 - T_1$ were determined. All statistical procedures were calculated by use of the GraphPad Prism® statistical software package, version 4.00 (GraphPad Software Inc.; San Diego, CA, U.S.A.). Data analysis was performed by one-way analysis of variance (ANOVA) with the Tukey test for multiple comparisons. Data are expressed as MEAN ± S.E.M. Statistical significance was set at $P < 0.05$.

Results

Exposure to an open field stress for 10 min increased body temperature in male C57BL/6J mice (Fig. **1**). Longer exposure times (30 min, 60 min) did not further enhance the temperature increase. Home cage animals did not show alterations in body temperature after 60 min (Fig. **1**, insert). Fig. **2** shows the effect of buspirone (10 mg/kg), fluoxetine (10 mg/kg), imipramine (20 mg/kg) and diazepam (5 mg/kg) on body temperature of C57BL/6J mice after 10 min of OF stress. Oral administration of buspirone significantly suppressed the elevation of body temperature induced by 10 min of OF stress, whereas oral administration of diazepam completely abolished SIH. Oral administration of the antidepressants imipramine and fluoxetine had no effect on SIH. To further validate the model we examined the effect of all positive controls on body temperature in unstressed mice in order to exclude possible hypo/hyperthermic effects of the compounds itself. Table **1** shows the effects of oral administration of all positive controls on changes in body temperature without exposure to OF stress. No changes in body temperature were observed after treatment with any of these substances. As shown in Fig. **2**, SJW extract dose dependently decreased ΔT. The optimum dose range was observed between 250 and 500 mg/kg. Interestingly, 250 mg/kg seemed to be more effective than 500 mg/kg. 125 mg/kg as well as 750 and 1000 mg/kg did not affect ΔT. SJW extract had no effect on body temperature after 60 min in unstressed mice (Table **2**). Fig. **3** shows the effect of various flavonoids from SJW in the SIH model. As shown in Fig. **3A**, hyperoside, isoquercitrin and quercitrin partially blocked OF induced hyperthermia in a concentration of 0.6 mg/kg. The effect was comparable to the 5-HT_{1A} receptor agonist buspirone (10 mg/kg). When administered in a higher dose (1.2 mg/kg), hyperoside and isoquercitrin remained active whereas quercitrin showed no effect on ΔT (Fig. **3B**). The quercetin 3-*O*-glucuronide miquelianin was inactive in a dosage of 0.6 mg/kg but significantly reduced ΔT in a concentration of 1.2 mg/kg (Fig. **3B**). Fig. **4A** shows the effect of rutin on SIH. Rutin partially reduced ΔT at 1 mg/kg, but had no effect at 2 mg/kg. From the biflavonoids tested, only amentoflavone in a concentration of 0.1 mg/kg significantly blocked ΔT (Fig. **4B**), whereas biapigenin (1 mg and 2 mg/kg) did not affect the elevated body temperature after

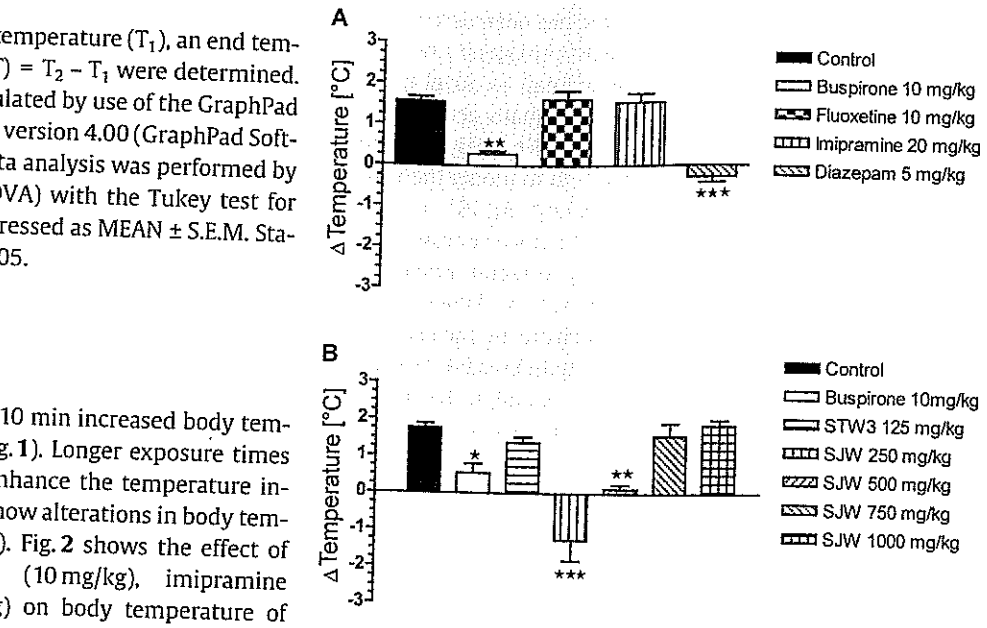

Fig. 2 Effect of buspirone, fluoxetine, imipramine, diazepam (**A**) and of SJW (**B**) on Δ after 10 min of open field stress. Values represent Mean ± SEM of n = 8 per group. * P < 0.05, ** P < 0.01, *** P < 0.001 as compared to control.

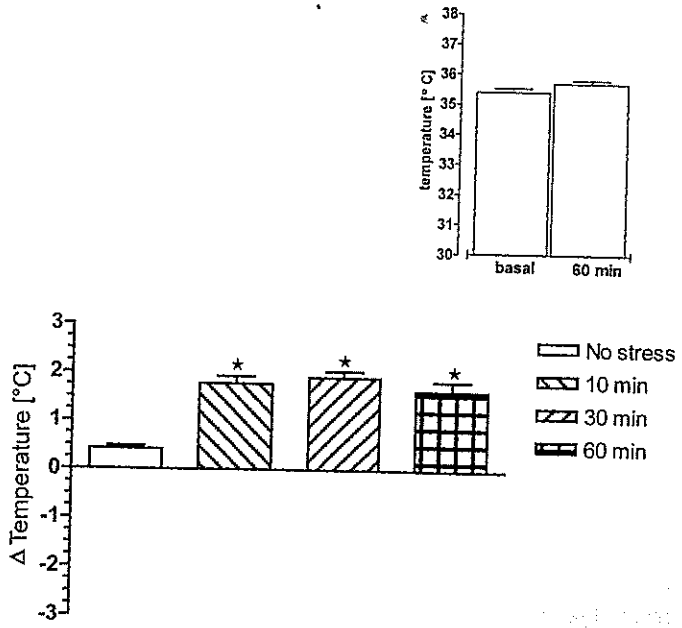

Fig. 1 Changes in Δ of male C57BL/6J mice exposed to 10, 30, or 60 min of open field stress. Control animals were not exposed to stress. Insert: Body temperature of unstressed rats after 60 min (= home cage animals). Values represent mean ± SEM of n = 8 per group. * P < 0.05 as compared to control.

Table 1 Effects of diazepam, buspirone, imipramine, and fluoxetine on changes in body temperature of unstressed C57BL/6J mice at t = 0 (basal, before oral treatment) and t = 60 min (after oral treatment). Compounds were given orally, data are expressed as mean ± SEM, n = 8 animals per group

Treatment	*Basal [°C]*	*1 h [°C]*
Control	36.5 ± 0.12	36.3 ± 0.08
Diazepam 5 mg/kg	36.6 ± 0.13	36.2 ± 0.18
Buspirone 10 mg/kg	36.4 ± 0.11	36.2 ± 0.09
Imipramine 20 mg/kg	36.3 ± 0.15	36.3 ± 0.15
Fluoxetine 10 mg/kg	36.4 ± 0.13	36.2 ± 0.14

Table 2 Effects of St. John's wort extract on changes in body temperature of unstressed C57BL/6J mice at t = 0 (basal, before oral treatment) and t = 60 min (after oral treatment). Compounds were given orally, data are expressed as mean ± SEM, n = 8 animals per group

Treatment	*Basal [°C]*	*1 h [°C]*
Control	36.3 ± 0.15	36.2 ± 0.16
STW3 VI 125 mg/kg	36.0 ± 0.20	36.2 ± 0.18
STW3 VI 250 mg/kg	36.1 ± 0.20	34.9 ± 0.40
STW3 VI 500 mg/kg	36.3 ± 0.17	36.1 ± 0.30

exposure to open field stress. As shown in Fig. **4C** hyperforin in a concentration range of 1–10 mg/kg had no effect on ΔT. The naphthodianthrone hypericin significantly reduced ΔT in a concentration of 0.1 mg/kg but had no effect at 0.5 mg/kg. None of the tested compounds affected body temperature of unstressed mice (Table **3**).

Discussion

When mammals, including man, are confronted with a stressful event their core body temperature rises, known as "stress-induced hyperthermia" (SIH). In mice, the SIH procedure has been developed to measure anti-stress or anxiolytic-like effects of psychoactive drugs [3]. SIH is mediated by the autonomic nervous system and occurs prior to and during exposure to anxiogenic or stress-inducing stimuli, like noise, heat, handling, novelty or pain [3], [23]. In many anxiety disorders, it occurs as an integral part of the pathology and is often considered a representative symptom of the disease [20]. In the present study, we assessed the effect of a variation of the original SIH-induced paradigm by placing the animal 60 min after oral application of the test compound into an open field [9], [10]. By this variation, psychological stress induced by the novel environment of the open field is thought to result in a more general and elevated form of anxiety. In our procedure, SIH (ΔT) is calculated as the difference between the basal T (before oral treatment) and the end T (after 10 min OF stress). The stress-induced rise in rectal temperature can be blocked by various mechanisms. SIH could specifically be prevented by prior treatment with drugs possessing anxiolytic activity. In this model, the benzodiazepine diazepam and the 5-HT_{1A}-receptor agonist buspirone were active to block SIH (Fig. **2**). In contrast to the diazepam, which completely counteracted SIH, buspirone only partially blocked SIH. This might point to differential mechanisms (benzodiazepine receptor agonists, 5-HT_{1A}-receptor agonists) of action which regulate SIH. However, our results are in good correlation with previously published data [20], [6]. The authors also speculate that the partial inhibition of SIH by 5-HT_{1A}-receptor agonists may reflect the intrinsic activity of these compounds at the 5-HT_{1A}-receptor. In these particular papers flesinoxan – a full 5-HT_{1A}-receptor agonist – completely blocked SIH whereas buspirone – a partial 5-HT_{1A}-receptor agonist – abolished the temperature increase after stress only to certain extents.

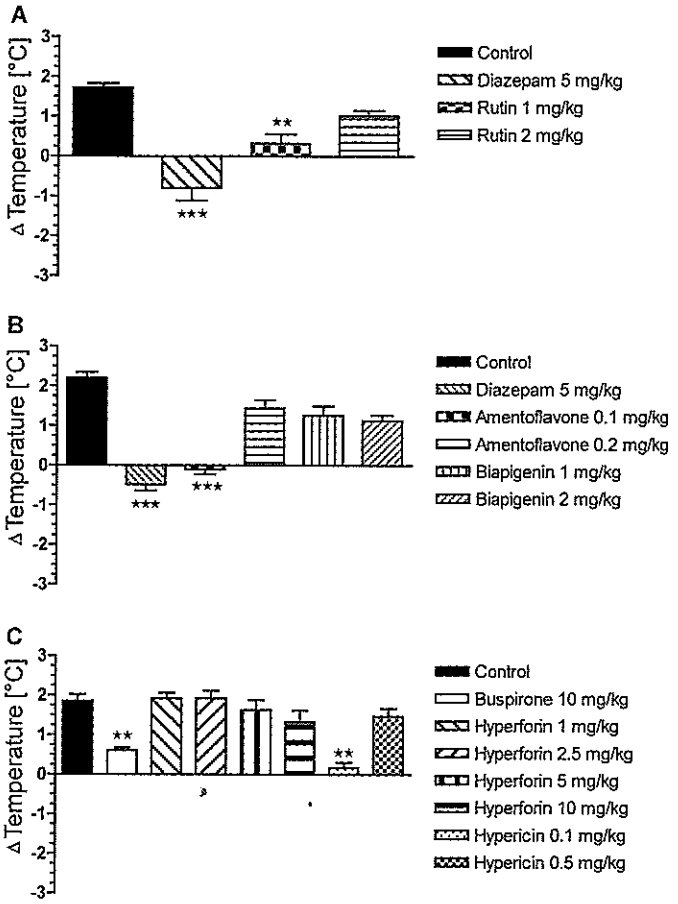

Fig. 4 Effect of rutin (**A**), amentoflavone and I3,II8-biapigenin (**B**), hyperforin and hypericin (**C**) on Δ after 10 min of open field stress. Values represent mean ± SEM of n = 8 per group. ** P < 0.01, *** P < 0.001 as compared to control.

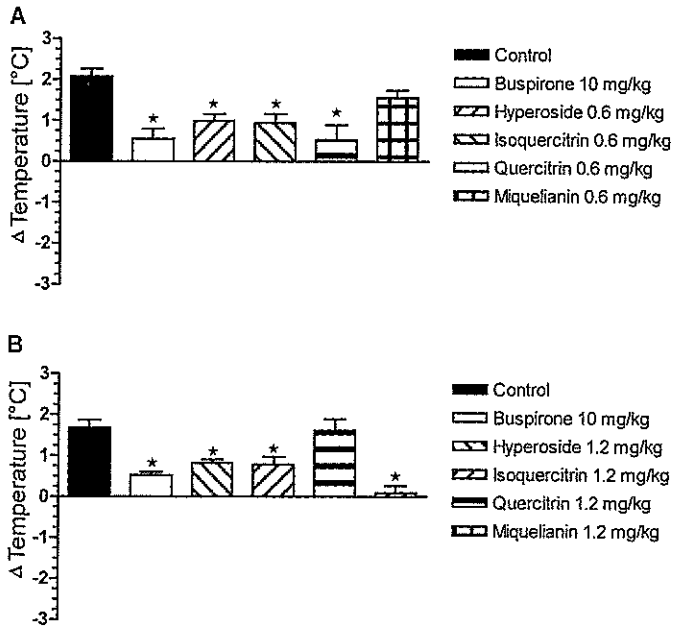

Fig. 3 Effect of various flavonoids on Δ after 10 min of open field stress. Values represent mean ± SEM of n = 8 per group. * P < 0.05 as compared to control.

Table 3 Effects of various flavonoids, the biflavonoids amentoflavone and I3,II8-biapigenin as well as hyperforin and hypericin on changes in body temperature of unstressed C57BL/6J mice at t = 0 (basal, before oral treatment) and t = 60 min (after oral treatment). Compounds were given orally, data are expressed as mean ± SEM, n = 8 animals per group

Treatment	*Basal [°C]*	*1 h[°C]*
Control	36.6 ± 0.16	36.4 ± 0.19
Hyperoside 0.6 mg/kg	36.3 ± 0.18	36.0 ± 0.19
Hyperoside 1.2 mg/kg	36.3 ± 0.20	36.5 ± 0.12
Isoquercitrin 0.6 mg/kg	36.2 ± 0.14	36.5 ± 0.14
Isoquercitrin 1.2 mg/kg	36.0 ± 0.18	36.2 ± 0.13
Quercitrin 0.6 mg/kg	36.2 ± 0.18	34.8 ± 0.25
Quercitrin 1.2 mg/kg	36.1 ± 0.17	36.2 ± 0.13
Miquelianin 0.6 mg/kg	36.1 ± 0.19	36.2 ± 0.17
Miquelianin 1.2 mg/kg	36.5 ± 0.19	36.7 ± 0.17
Rutin 1 mg/kg	36.3 ± 0.10	36.0 ± 0.13
Rutin 2 mg/kg	36.5 ± 0.13	36.2 ± 0.16
Amentoflavone 0.1 mg/kg	36.3 ± 0.23	36.5 ± 0.25
Amentoflavone 0.2 mg/kg	36.6 ± 0.24	36.3 ± 0.19
Biapigenin 1 mg/kg	36.7 ± 0.32	36.3 ± 0.13
Biapigenin 2 mg/kg	36.0 ± 0.13	36.1 ± 0.14
Hyperforin 5 mg/kg	36.3 ± 0.23	36.5 ± 0.25
Hyperforin 10 mg/kg	36.4 ± 0.21	36.1 ± 0.19
Hypericin 0.1 mg/kg	36.6 ± 0.17	36.4 ± 0.14
Hypericin 0.5 mg/kg	36.4 ± 0.14	36.7 ± 0.20

Antidepressants were unable to block the SIH. The mixed noradrenergic/serotonergic re-uptake inhibitor imipramine and the selective-serotonin re-uptake inhibitor fluoxetine did not antagonize SIH (Fig. 2). Our results are in line with earlier findings [20], [6] and suggest that enhancement of monoaminergic transmission does not play an important role in the antagonism of SIH.

In the present experiments, the SJW extract completely blocked SIH in a concentration of 250 mg/kg. In a concentration of 500 mg/kg the extract also significantly reduced ΔT but to a lower extent. Concentrations below 250 mg/kg and above 500 mg/kg were inactive, indicating a U-shaped dose-response curve of the extract (Fig. 2). The occurrence of U-shaped dose-responses are a widely and independently observed phenomenon [24]. Yet, despite the widespread nature of their occurrence, little attempt has been made to assess U-shaped dose-responses as integrative phenomena. Instead they are regarded as a string of apparently reproducible, but biologically unrelated, responses [24]. However, this suggests that the widespread occurrence of these U-shaped dose-responses might be examples of biological optimization processes [24].

From the flavonoids tested in the present experiments all of them only partially blocked SIH (Fig. 3). Hyperoside, isoquercitrin and quercitrin decreased ΔT in a concentration of 0.6 mg/kg, miquelianin in a dosage of 1.2 mg/kg and rutin at 1 mg/kg. The doses used for the present experiments have shown to be active in previous experiments using the forced swimming test [22]. The reduction of ΔT for this class of compounds was comparable to the 5-HT_{1A}-receptor agonist buspirone, which also partially but not completely blocked SIH. This might point to a similar mechanism regulating SIH, but this remains speculative at present.

The most potent effect on SIH was seen for the biflavonoid amentoflavone (0.1 mg/kg; this amount of amentoflavone is present in 250 mg/kg dosage) with similar activities on SIH as the benzodiazepine diazepam. Interestingly, I3,II-8-biapigenin, which is structurally similar to amentoflavone showed no activity in the doses tested (biapigenin was tested in a dose 10-fold higher than that of amentoflavone because this reflects their quantitative ratio in the plant). There is *in vitro* evidence suggesting that some flavonoids, including amentoflavone may elicit a sedative effect that could involve both benzodiazepine and GABA receptor agonism [25], [26], [27]. In our previous *in vitro* investigations, the biflavonoid amentoflavone showed the most interesting receptor binding profile compared to the other tested flavonoids showing high affinities to the rat benzodiazepine receptor [28]. Together with the results from *in vitro* studies it can be concluded that the SIH and therefore the anxiolytic effects of amentoflavone are likely to be mediated via the benzodiazepine receptor.

The phloroglucinol derivative hyperforin did not show anxiolytic properties in our SIH model (Fig. 4). In a previous study, Zanoli et al. investigated a newly synthesized, more stable and therefore more bioavailable derivative, hyperforin acetate in several behavioral models [29]. The authors could show that in the elevated plus-maze and in the light-dark test, the acute administration of hyperforin acetate (3–5 mg/kg) exerted an anxiolytic activity, which, however, was smaller than that of diazepam [29]. Thus, it can be speculated that the lack of effect of hyperforin in the present study might be due to bioavailability issues.

The naphthodianthrone hypericin decreased ΔT in a concentration of 0.1 mg/kg; the higher dose of 0.5 mg/kg was inactive (Fig. 4). The effect of hypericin was slightly more pronounced than that of buspirone, but the naphthodianthrone was not as active as diazepam. The concentration of hypericin used in the present study has been shown to be active in our previous experiments [30]. The mechanism behind the activity of hypericin in this model, however, remains speculative. Therefore, further experiments are needed, especially behavioral experiments to investigate the anxiolytic activity of hypericin.

In conclusion, using open field stress as a psychological stressor to induce hyperthermia in mice we were able to detect putative anxiolytic effects of SJW extract and single compounds. The anxiolytic properties apparently were not limited to one mechanism of action. In this model, both positive controls, the benzodiazepine diazepam and the 5-HT_{1A} receptor agonist buspirone, exerted anxiolytic activity. These results are consistent with previously published data [6], [20]. The anxiolytic activity of SJW extract in the present experiments was not attributed to a single active compound. In fact, we could show that several constituents affected SIH in a diverse manner. Most active of all tested compounds, however, was the biflavonoid amentoflavone, which is present in low, but sufficient concentrations in SWJ extracts [21]. Further behavioral and mechanistic studies are needed to establish the pharmacological relevance of these findings for the therapeutic usage of SJW.

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