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Thermoregulatory Effects of Alkaloids Isolated From Wu-Chu-Yu in Afebrile and Febrile Rats

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TSAI, T. H., T. F. LEE, C. F. CHEN AND L. C. H. WANG. *Thermoregulatory effects of alkaloids isolated from Wu-chu-yu in afebrile and febrile rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 293–298, 1995.—Dehydroevodiamine (DeHE) and evodiamine (EVO), alkaloids isolated from a Chinese medicinal herb, Wu-chu-yu, exhibit calcium antagonistic activity. Intraperitoneal injections of DeHE (5–20 mg/kg) and EVO (2.5–10 mg/kg) caused a dose-related hypothermia in afebrile rats at an ambient temperature (Ta) of 20°C. Because the heat production of alkaloid-injected rats did not differ from that of the controls, the hypothermic effect likely resulted from increased peripheral heat loss. This suggestion is supported by the finding that both DeHE and EVO did not affect the thermoregulatory response of rats exposed to a Ta of 35°C, at which heat loss was maximized. Injection of the same doses of DeHE and EVO attenuated the febrile response in a dose-related manner, induced by intrahypothalamic injection of exogenous pyrogen. The attenuation of the febrile response was associated with a reduction in heat production. Because DeHE and EVO did not affect HP in afebrile rats at a Ta of either 20 or 35°C, but suppressed the metabolic rate of febrile rats at 20°C, the thermoregulatory effect of DeHE and EVO could involve both a calcium-dependent increase in heat loss and a suppression in heat production; the latter may only be manifested when the set point for thermoregulation is elevated.

Dehydroevodiamine Evodiamine Calcium Calcium channel blocker Fever Thermoregulation

OVER the last 2 decades, there has been accumulating pharmacologic and physiologic evidence that endogenous Ca^{2+} ions may play a functional role in determining set point in thermoregulation (8,9). Intracerebroventricular (ICV) perfusion of excess Ca^{2+} ions causes a fall in body temperature (Tb), whereas a reduction of brain Ca^{2+} level by chelation with EGTA in the perfusion fluid elicits a rise in Tb in a variety of species. In subsequent studies, the posterior hypothalamus has been demonstrated to be the anatomic site at which Ca^{2+} ions elicit the thermolytic effect (8,9): an increase and decrease in $^{45}Ca^{2+}$ efflux from the posterior hypothalamus was observed when cats were exposed to cold and heat, respectively. Furthermore, the efflux of Ca^{2+} ions from the posterior hypothalamus of cats was significantly augmented when their Tb was elevated following ICV infusion of pyrogen. Pretreating the animal with antipyretic significantly reduced the efflux of Ca^{2+} ions (8,9). Taken together, this evidence indicates that Ca^{2+} ions within the posterior hypothalamus play a vital role in governing thermoregulation not only in a normal state, but also in a pathophysiologic state such as fever.

Dehydroevodiamine (DeHE) and evodiamine (EVO), alkaloids isolated and purified from Wu-chu-yu (the dry unripe fruits of *Evodia rutaecarpa*, a longstanding Chinese medicinal plant) have been shown to exhibit calcium antagonistic activity. Both DeHE (18) and EVO (4) can reduce the vascular contractile response induced by either $CaCl_2$ or phenylephrine (via Ca^{2+} influx). Further studies also showed that YC-08, a derivative of DeHE, can inhibit the intracellular Ca^{2+} rise in Fura-2-loaded platelets stimulated by collagen (19). As described earlier, altering the level of posterior hypothalamic Ca^{2+} has a profound effect on shifting the thermoregulatory set point. The administration of DeHE and EVO, acting as calcium antagonists, is thus expected to induce various thermoregulatory changes. Even though the dose and route of administration were not described, the crude alcohol extract of Wu-chu-yu has been shown to elevated the Tb of the rabbit (3). It is therefore interesting to examine whether DeHE and EVO could account for this thermoregulatory effect under both normal (afebrile) and pathophysiologic (febrile) conditions in rats.

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METHOD

All experimental protocols were approved by the University of Alberta Animal Care Committee, following the guidelines of the Canadian Council on Animal Care. Adult Sprague-Dawley rats (3–6 mo) were used in the present study. The rats were kept individually at 20°C and in a 12 L : 12 D photoperiod. After the body weight of rats reached about 400 g, rodent chow was rationed (about 20 g/day) throughout the whole experimental period to maintain constant weight and minimize variation in results resulting from differences in body size. Water was available at all times.

Stereotaxic Procedure

Under halothane anesthesia, a guide cannula (23-ga stainless-steel tubing) was implanted unilaterally into the preoptic anterior hypothalamus (POAH) at the following stereotaxic coordinate: AP = 7.8 mm, L = 1.0 mm, H = 8.0 mm below the dura matter (10). The tip of each guide tube was beveled and positioned 1.0 mm above the injection site to minimize damage to the actual injection site. After completion of the experiments, the precise anatomic location of the injection site was subsequently verified histologically.

Body Temperature and Oxygen Consumption Measurement

One week after the operation, each rat was transferred to a circular, Plexiglas water-jacketed metabolism chamber (20 × 20 cm, diameter × height) in which the ambient temperature (Ta) could be controlled accurately at 20 ± 1°C. The Tb of the rat was recorded continuously with a precalibrated temperature-sensitive radiotransmitter (model T-M; Mini- Mitter Co.), which was implanted in the peritoneal cavity at the same time as the guide cannula. Exhaust gas from the metabolism chamber was divided into two streams: one for O_2 measurement (Applied Electrochemistry O_2 analyzer, S-3A/II) after desiccation with Drierite and CO_2 removal with Ascarite, and the other for measurement of CO_2 after desiccation (Applied Electrochemistry CO_2 analyzer, CD-3A). Using an IBM personal computer interfaced with an ISAAC 91-I data acquisition system, oxygen consumption and CO_2 production were recorded and integrated simultaneously with the Tb transmitter signal. Heat production (HP) was calculated from oxygen consumption and the respiratory quotient (16), and the Tb was derived based on a precalibration curve established for each individual transmitter. In the heat-exposure experiment, rats were placed in the test chamber maintained initially at 20°C to measure their baseline Tb and HP. After attaining stable Tb and HP, the Ta was increased to 35 ± 1°C and the rats were kept at that temperature for another 2 h.

Drugs Used and Data Analysis

Lipopolysaccharides (LPS) extracted from *Salmonella typhosa* were purchased from Sigma Chemical Co. (St. Louis, MO). Both DeHE and EVO were obtained from the dried fruit of *Evodiae rutaecarpa*, first extracted with ethanol (60°C for 16 h, four times), then separated by column chromatography (Amberlite XAD-2). The purity of both compounds was determined by high performance liquid chromatography and was found to be > 99%. All compounds except the LPS were dissolved in dimethyl sulphoxide (DMSO) immediately before the experiment, and all test solutions were passed through a 0.22 μm Swinnex Millipore filter to ensure sterility. The LPS was prepared in sterile artificial cerebrospinal fluid. To examine the effectiveness of the compounds on thermoregulatory responses to varying Ta, either DMSO (control vehicle), DeHE, or EVO was injected intraperitoneally (IP) in a volume of 1 ml/kg. Immediately afterward, Ta was increased to 35°C heat exposure. For pyrogen-induced fever, the LPS (2.5 μg/μl) was injected via the POAH cannula 30 min after DMSO, DeHE, or EVO injection.

Statistical analysis of the thermoregulatory effects of the alkaloids was by multivariate analysis of variance (SPSS-PC; SPSS Inc.). Significance was set at $p < 0.05$, unless otherwise stated.

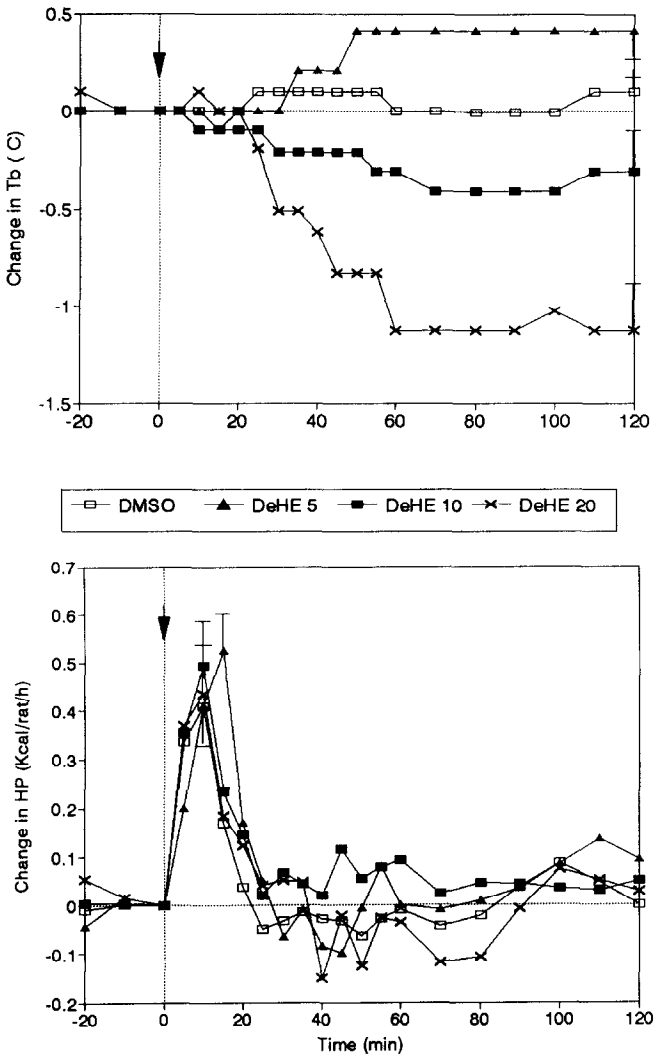

FIG. 1. Time course of change in core temperature (top panel) and heat production (bottom panel) after IP injection of DMSO (□), DeHE 5 (▲), 10 (■), or 20 mg/kg (×) in rats kept at 20°C. Each point represents the mean change from seven rats. The SEM is represented by a vertical bar and is shown only at the peak response value of each dose for clarity. The arrow indicates the time of injection.

RESULTS

Thermoregulatory Effects of DeHE and EVO in Afebrile Rats

Control rats exposed to 20°C had an initial mean Tb of 37.4 ± 0.16°C and a resting HP of 1.38 ± 0.06 kcal/h per

animal (5.77 ± 0.25 KJ/h per animal) (n = 7). As shown in Fig. 1, IP administration of DMSO caused slight increases in Tb and HP, which were not significantly different from the preinjection values. In contrast, injection of DeHE (5–20 mg/kg) induced dose-related hypothermia [$F(28, 168) = 2.59$, $p < 0.001$] (Fig. 1). Regardless of the dosage used, however, the change in HP after DeHE injection was similar to that of the controls (Fig. 1). The thermoregulatory response of rats to IP injection of EVO (2.5–10 mg/kg) was similar to that after DeHE injection (Fig. 2), but the minimal dose of EVO (2.5 mg/kg) required to elicit significant hypothermia [$F(14, 84) = 2.24$, $p < 0.05$] was only about a quarter of that for DeHE (10 mg/kg). In most cases, especially after receiving the highest dose of either DeHE or EVO, the rat lay spread out on the floor of the metabolism chamber for the first 20–30 min.

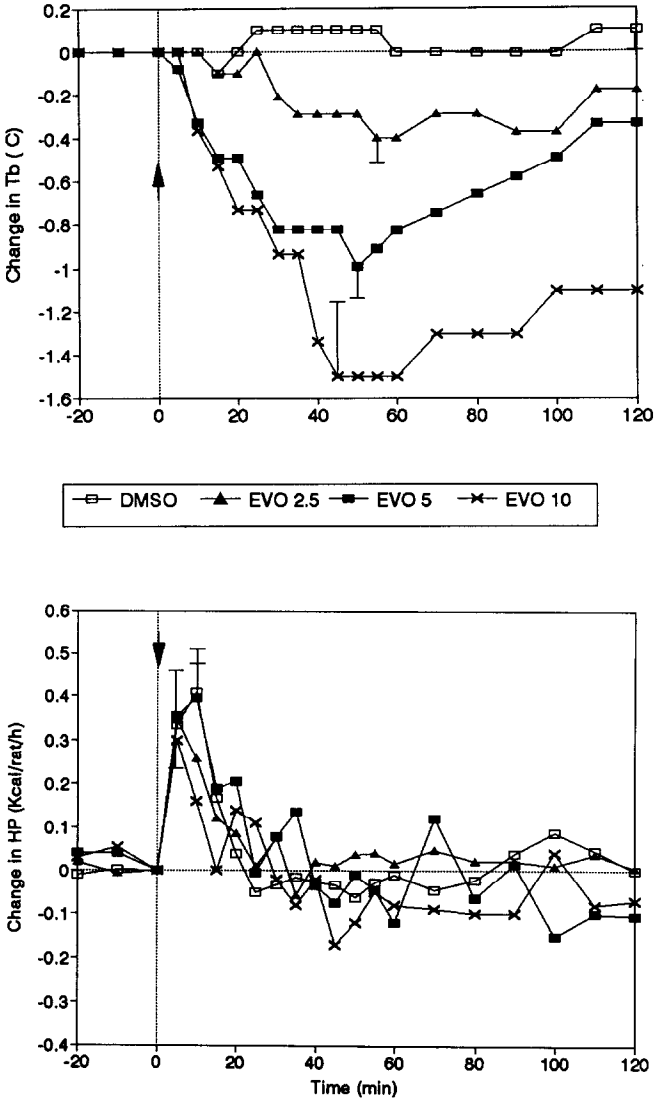

FIG. 2. Time course of change in core temperature (top panel) and heat production (bottom panel) after IP injection of DMSO (□), EVO 2.5 (▲), 5 (■) or 10 mg/kg (×) in rats kept at 20°C. Each point represents the mean change from seven rats. The SEM is represented by a vertical bar and is shown only at the peak response value of each dose for clarity. The arrow indicates the time of injection.

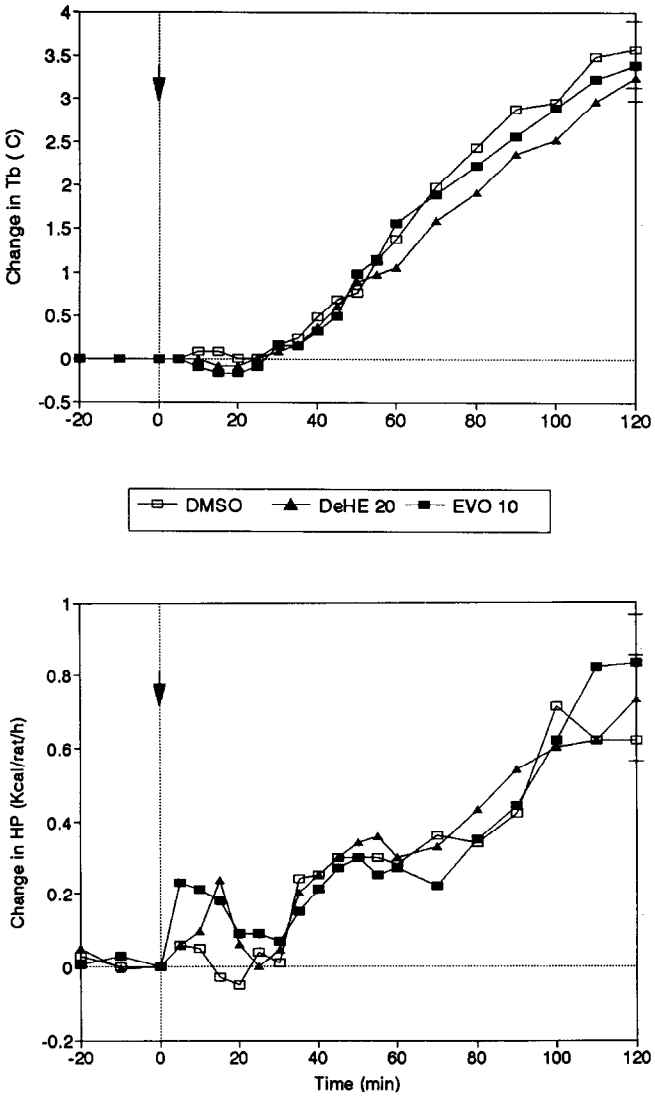

FIG. 3. Time course of change in core temperature (top panel) and heat production (bottom panel) after IP injection of DMSO (□), DeHE 20 (▲), or EVO 10 mg/kg (■) in rats under heat stress. Each point represents the mean change from seven rats. The SEM is represented by a vertical bar and is shown only at the peak response value of each dose for clarity. The arrow indicates the time of injection and change in chamber temperature to 35°C.

Thereafter, the animal returned to normal posture and displayed a slight increase in activity (grooming and licking) throughout the rest of the experimental period.

Effects of DeHE and EVO on Thermoregulatory Responses Under Heat Exposure

To examine whether the temperature changes observed after DeHE and EVO administration resulted from autonomic side-effects, their effects on temperature regulation in rats under heat stress (35°C) were examined; the results are summarized in Fig. 3. In rats pretreated with DMSO, there was no significant change in both Tb and HP within the first 30 min after the Ta was raised. Thereafter, both the Tb and HP rose steadily and significant hyperthermia (+3.5°C) was observed

after 120 min. Injection of the highest dose of DeHE (20 mg/kg) or EVO (10 mg/kg) did not affect the pattern of hyperthermia development under heat exposure (Fig. 3), suggesting that the alkaloid-induced thermoregulatory changes observed in rats kept at at 20°C was not due to any impairment of thermoregulatory functions of the animal.

Effects of DeHE and EVO on Pyrogen-Induced Fever

To examine the thermoregulatory effects of DeHE and EVO under pathophysiologic conditions in rats, a febrile state was induced by POAH injection of LPS extracted from *S. typhosa* (2.5 μg). Similar to previous results (7), an intrahypothalamic injection of LPS induced long-lasting fever that was associated with an increase in HP. Pretreatment with either IP DeHE (Fig. 4) [$F(28, 168) = 5.85$, $p < 0.001$] or EVO (Fig. 5) [$F(28, 168) = 2.43$, $p < 0.001$] attenuated the LPS-induced fever in a dose-related manner. Furthermore, the magnitude of LPS-induced HP was also significantly attenuated after either DeHE (Fig. 4) or EVO pretreatment (Fig. 5).

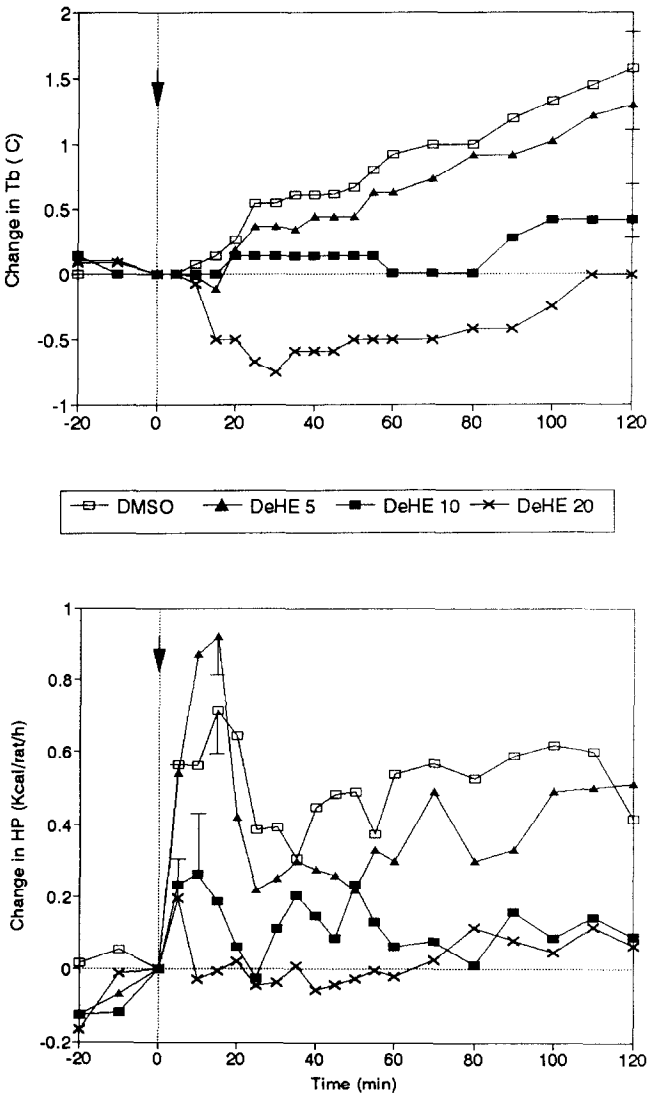

FIG. 4. Time course of change in core temperature (top panel) and heat production (bottom panel) after IP injection of DMSO (□), DeHE 5 (▲), 10 (■), or 20 mg/kg (×) in febrile rats kept at 20°C. Each point represents the mean change from seven rats. The SEM is represented by a vertical bar and is shown only at the peak response value of each dose for clarity. The arrow indicates the time of intrahypothalamic LPS (2.5 μg) injection, and DMSO and various doses of DeHE were pretreated 30 min before LPS injection.

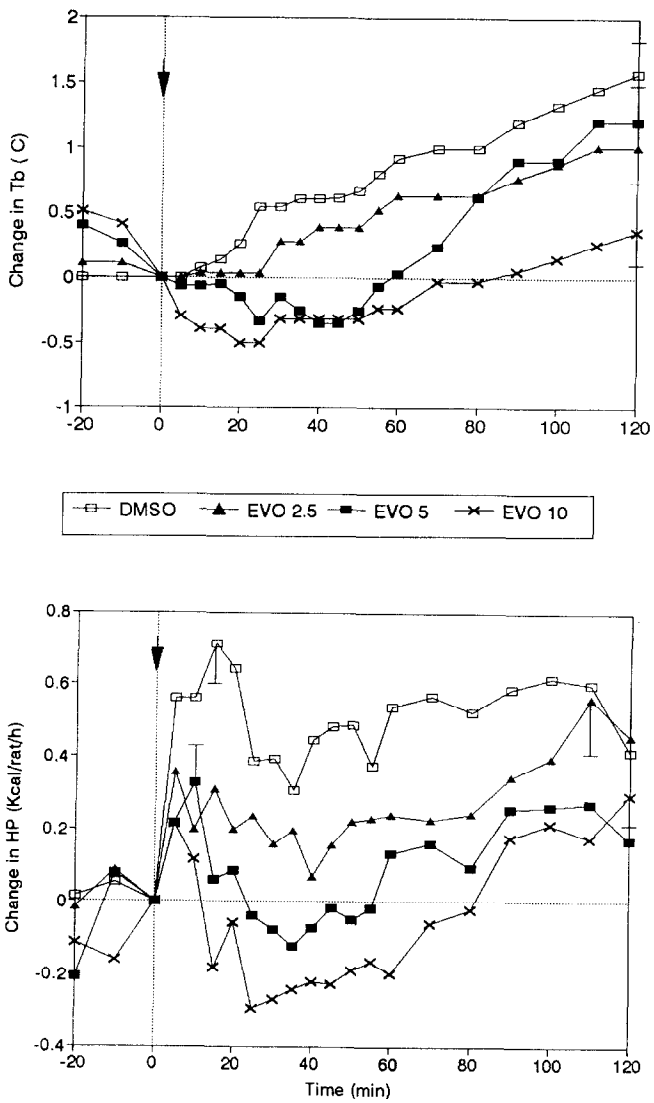

FIG. 5. Time course of change in core temperature (top panel) and heat production (bottom panel) after IP injection of DMSO (□), EVO 2.5 (▲), 5 (■), or 10 mg/kg (×) in febrile rats kept at 20°C. Each point represents the mean change from seven rats. The SEM is represented by a vertical bar and is shown only at the peak response value of each dose for clarity. Arrow indicates the time of intrahypothalamic LPS (2.5 μg) injection, and DMSO and various doses of DeHE were pretreated 30 min before LPS injection.

DISCUSSION

Intraperitoneal injections of DeHE and EVO, alkaloids isolated from Wu-chu-yu and possessing calcium antagonistic activity, caused a dose-related fall in Tb in afebrile rats ex-

posed to a Ta of 20°C. Our observed hypothermic response was similar to that seen after systemic injections of classic Ca^{2+} antagonists in animals kept at room temperature (2) or in the cold (6). Furthermore, the relative potency of DeHE and EVO in inducing hypothermia in vivo also reflects their relative potency in vitro as Ca^{2+} antagonists (17,18). These observations thus suggest that DeHE and EVO could have elicited their hypothermic effect through their Ca^{2+} channel-blocking activities. Although many studies have reported changes in Tb after either central or peripheral injection of Ca^{2+} channel blockers (1,2), only a few (6) examined the underlying mechanism(s). Based on the lack of change in HP (Figs. 1 and 2), increased heat loss is likely to be responsible for the observed alkaloid-induced hypothermia. This deduction is also supported by the observed behavioral changes (prone body extension and grooming), which were similar to the classic heat dissipation response elicited during heat exposure (12,13). A further indication that enhanced heat loss is involved in alkaloid-induced hypothermia was provided by the heat exposure study. Under heat exposure, whereas the heat loss was already maximized, pretreatment of alkaloids failed to elicit changes in the Tb of rats. As Ca^{2+} channel blockers, DeHE and EVO may prevent Ca^{2+} influx into the vascular smooth muscle, thereby reducing vasoconstriction and heat conservation. At a Ta of 20°C, which is below thermoneutrality for rats, this leads to increased heat loss and hypothermia. Because the alkaloid-treated rats responded identically to the control rats in both HP and Tb changes under heat stress, and because the HP of alkaloid-treated rats at 20°C did not differ from that of controls when thermogenesis was required below thermoneutrality, it is apparent that the alkaloids do not exhibit nonspecific impairment on general autonomic functions involved in thermoregulation.

It is well documented that Ca^{2+} ions within the posterior hypothalamus play an important role in regulating the thermoregulatory set point during febrile response (8,9); therefore, it is interesting to examine the thermoregulatory effects of DeHE and EVO under this pathophysiologic condition. Systemic injections of DeHE and EVO caused a dose-related attenuation of the febrile response to intrahypothalamic injections of exogenous pyrogen, which was associated with a reduction in HP. Because the rises in Tb and HP under simple heat stress were not affected by the alkaloids (DeHE 20 mg/g and EVO 10 mg/kg) (Fig. 3), and because the underlying mechanism (i.e., the reduction in HP) that caused the thermoregulatory effects of the alkaloids in the febrile rats appears to differ from that observed in the afebrile rats, the attenuation of the fever and HP is likely due to a specific, state-dependent effect of the alkaloids. In addition to attenuating HP, the possibility that these alkaloids also elicited increased HL in their fever-reducing effect cannot be rule out.

Because both DeHE and EVO were injected systemically, the exact mechanism(s) by which the alkaloids elicit their thermoregulatory effects remains unknown. It is possible that these alkaloids may elicit their effects by directly affecting the thermoregulatory effectors. However, it has been shown previously that Ca^{2+} channel blockers, when injected IP, can reduce opiate-induced hyperthermia by preventing the inhibitory effect of opiates on Ca^{2+}/Mg^{2+} ATPase activity in the hypothalamus (11). In the case of the present alkaloids, it has been shown that they are present in various brain tissues after systemic administration (Tsai and Chen, unpublished observation). It is therefore possible that the thermoregulatory effect of DeHE and EVO is the result of an alteration of Ca^{2+} transport, which in turn could alter the set point for thermoregulation. Recent evidence indicated that Ca^{2+} channel blockers can prevent a febrile response by inhibiting the Ca^{2+}-dependent activation of phospholipase A_2 as the release of intracellular Ca^{2+} (14,15), which is essential for the conversion of phospholipids into the arachidonic acid-prostaglandin cascade (5). Thus, as Ca^{2+} channel blockers, DeHE and EVO could also attenuate the febrile response by this later mechanism. Studies involving direct injection of the alkaloids into the posterior hypothalamic region in both afebrile and febrile rats can further clarify how DeHE and EVO affect thermoregulation under these different pathophysiologic states.

ACKNOWLEDGEMENT

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