Pyrogenicity of Etiocholanolone and Interleukin-1 in New and Old World Monkeys (44253)

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Abstract. **Etiocholanolone (5β-androstan-3α-ol-17-one; designated E) is one of the major products of metabolism of testosterone and androstenedione (androst-4-ene-3,17-dione) in many mammalian species, including humans. E and several other 5β-reduced steroids have been found to induce fever in humans. The pyrogenic effect of these steroids has been shown to be due to the release of interleukin-1 (IL-1) from the leukocytes that are mobilized in response to the steroid injections.**

Old World Monkeys such as Rhesus monkeys (*Macaca mulatta*), metabolize androgens similarly to humans, and E is a normal metabolite. However, New World Monkeys such as Squirrel monkeys (*Saimiri sciureus*), lack hepatic 5α- and 5β-steroid reductases and excrete androgens primarily in an unaltered state; E is not produced. Therefore, we postulate that Squirrel monkeys likewise may have lost the ability to respond to 17-ketosteroids such as E.

To test this hypothesis, adult male Rhesus and Squirrel monkeys were treated with E, and their rectal temperatures were recorded over a 24-hr period. Rhesus monkeys exhibited a rise of up to 3° F following E injection. Squirrel monkeys, on the other hand, did not exhibit any increase in rectal temperature over the 24-hr period, even when doses up to 250 times the effective human dose were used. However, both species responded to injected IL-1α with a robust increase in rectal temperature.

The data show that E is pyrogenic in Rhesus, but not Squirrel monkeys. The findings support the notion that injected E may induce release of IL-1 in Rhesus monkeys, but not in Squirrel monkeys. [P.S.E.B.M. 1998, Vol 217]

Etiocholanolone, (5β-androstan-3α-ol-17-one; designated E) and its optical isomer, androsterone (5α-androstan-3α-ol-17-one; A) are intermediary metabolites of testosterone (T) and androst-4-ene-3,17-dione (androstenedione; AD). AD and T (after 17-oxidation to AD) are reduced by liver 5α- and 5β-reductases to form A and E respectively. Rather than simply playing a passive role in steroid metabolism, both E and A can exert significant biological activities before being further reduced, conjugated, and excreted in the urine and bile.

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E is one of the few steroids known to induce fever in humans (1). The pyrogenic effect of E has since been shown to be mediated by interleukin-1 (IL-1), a cytokine well characterized as an endogenous pyrogen (see Refs. 2–5). E is thought to stimulate production and release of IL-1 from leukocytes, and the cytokine then travels to the anterior hypothalamus where it raises the "thermostat" of the thermoregulatory center (5).

A, on the other hand, is known to lower plasma cholesterol, and may be a significant factor in mediating the hypocholesterolemic effects of thyroid hormones (6–9). A has not been reported to affect body temperature.

Previous studies in experimental animals showed that substances that favored the production of E by the liver (enhanced 5β-reductase or inhibited 5α-reductase) induced elevated serum cholesterol (10, 11). We postulated that IL-1 might mediate the hypercholesterolemic effects of E as it does its pyrogenic effects.

To test this hypothesis, E was injected into Squirrel monkeys in order to observe the (postulated) effects of IL-1

on lipid metabolism in this species. The Squirrel monkey was chosen because it lacks the hepatic enzymes (5α- and 5β-reductases) that reduce testosterone to E, and thus there would be no E produced from endogenous sources to obfuscate the effects of the injected E (12, 13). The injected E had no effect on serum cholesterol in Squirrel monkeys. However, to our surprise, there also was no increase in body temperature in the E-injected Squirrel monkeys, suggesting that E was having no effect at all in this species. In fact, E elicited no pyrogenic effect in Squirrel monkeys at doses 10, 100, or 250 times those reported to induce IL-1 release and fever in humans (4). These results implied that no IL-1 had been released by E in the Squirrel monkeys.

Thus, the possibility exists that New World Monkeys, characterized by the inability to metabolize androgens *via* the 5α- and 5β-reductase pathways, may also have lost the ability to respond to the biological effects of the 17-ketosteroid metabolites that are produced by these pathways. It would be even more surprising if New World Monkeys did not show a pyrogenic response to IL-1.

Materials and Methods

The experiments were approved by the LEMSIP Institutional Animal Care and Use Committee. Eight healthy young adult male Squirrel monkeys (*Saimiri sciureus*) 3–4 years old and weighing 700–800 g were purchased from Charles River Primate Imports, quarantined for 90 days, and conditioned for an additional 3 months. The animals were fed a diet consisting of two biscuits (30.4 g) of Purina High Protein Monkey Chow (Purina Mills, Inc., Richmond, IN) and 60 g canned Zu/Preem Marmoset Diet (Hills Pet Products, Topeka, KS) daily. The biscuits were fed in the morning and the canned diet (as slices) in the afternoon. In addition, the monkeys received small amounts of fresh fruit daily.

Eight healthy adult male Rhesus monkeys (*Macaca mulatta*) of the LEMSIP colony weighing 5.3–9.2 kg were fed *ad libitum* Monkey Diet biscuits (PMI Feeds, Inc., Richmond, IN) for Old World primates, supplemented daily with small amounts of fresh fruit.

Etiocholanolone (E) and its stereoisomer, androsterone (A; chosen as a control steroid) were purchased from Steraloids, Inc. (Wilton, NH). The steroids were dissolved in dimethylsulfoxide (Sigma Chemical Co., St Louis, MO) and sterile filtered. On the days of the experiment the steroids were injected in 0.1 ml vols into the thigh muscle of each animal. In preliminary studies, groups of two Squirrel monkeys each were injected with E or A in doses of 1, 10, or 25 mg/kg. As none of the doses raised rectal temperature, only the 25 mg/kg dose was used in subsequent experiments, in which an additional four monkeys each were injected with E or A. The data from the two experiments were combined, giving an n of 6 for the 25 mg/kg doses of E and A.

Rhesus monkeys were injected with 1 mg/kg E or A in two separate experiments, giving a final n of 8 for E and 5 for A.

Recombinant human interleukin-1α (IL-1α) was purchased from Bachem Bioscience Inc. (Philadelphia, PA), diluted with sterile, pyrogen-free 0.15 *M* NaCl, and injected iv (saphenous vein) in three Rhesus monkeys and three Squirrel monkeys at a dose of 30 ng/kg. Three control monkeys of each species received an iv injection of sterile, pyrogen-free 0.15 *M* NaCl.

Body temperatures were taken with a rectal thermal probe (Becton Dickinson, Franklin Lakes, NJ). It is important to note that both species required 3–4 weeks acclimation to the procedures of capture, restraint, injection, and insertion of the rectal temperature probe before reproducible body temperatures could be obtained. In general, the rectal temperatures observed were in the range reported in the literature for these species (92.9°–102.5°F for Squirrel monkeys and 96.8°–104.0°F for Rhesus monkeys; 14).

During the actual experiments, the monkeys were injected im with E or A, and then the body temperatures were recorded at 0, 30, and 60 min, and 2, 4, 6, and 24 hr in Rhesus monkeys and 0, 1, 3, 6, and 24 hr in Squirrel monkeys. Following iv IL-1α injection, body temperatures were recorded at 30, 60, and 120 min.

Data were analyzed using the statistical software, Instat (Graphpad Software, Inc., San Diego, CA). Data were calculated as change (in °F) from initial body temperature and plotted versus time after injection. Mean rectal temperatures for each group (A versus E or IL-1α versus saline) were also compared at each time interval. Significance of differences was determined by ANOVA and two-tailed t test. Statistical comparisons of actual temperature data were made after their conversion to natural logarithms (ln). Statistical evaluation of changes (up or down) from initial (preinjection) temperatures were made by testing the significance of the mean change from the theoretical value of zero (0 = no change) at each time point. Significance of differences was set at $p = 0.05$.

Results

Subcutaneous injections of E at doses of 1, 10, or 25 mg/kg had no consistent effect on rectal temperature of Squirrel monkeys over a 24-hr period. The nonpyrogenic steroid, A, likewise did not significantly alter body temperature over the 24-hr span. The results obtained with the highest doses of E and A (25 mg/kg) are shown in Figure 1. In contrast, injections of 1.0 mg/kg E in Rhesus monkeys resulted in a clearcut thermogenic effect that was not seen with the same dose of A in this species (Fig. 2). The mean increases in rectal temperature differed significantly from the mean Time 0 temperatures of the same monkeys at both 6 hr ($P < 0.05$) and 24 hr ($P < 0.001$). The fluctuations of the rectal temperatures of the A-treated Rhesus monkeys from their initial temperatures were insignificant ($P > 0.05$).

The 24-hr data for Squirrel and Rhesus monkeys are compared in Figure 3. A clearcut elevation in rectal temperature occurred only in the E-injected Rhesus monkeys (P

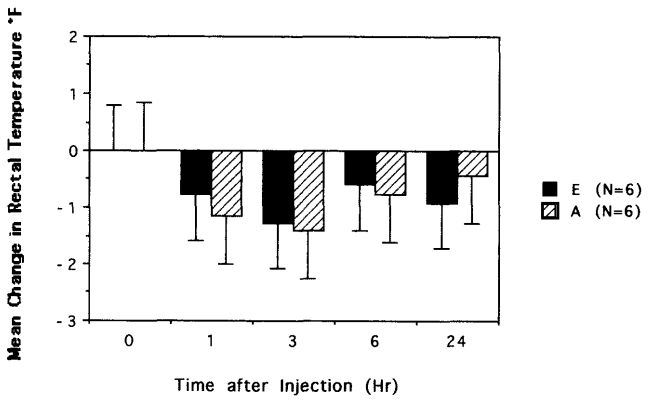

Figure 1. Effects of etiocholanolone (E) and androsterone (A) on rectal temperature in Squirrel monkeys. Each steroid was injected sc at a dose of 25 mg/kg into six monkeys at Time 0. Zero time temperatures (°F ± SD) were 105.0 ± 0.85 for E and 104.5 ± 0.79 for A. The error bars are the standard deviations. None of the means differed significantly from that of the zero time point ($P > 0.05$). The means for E did not differ from those for A at any time ($P > 0.05$).

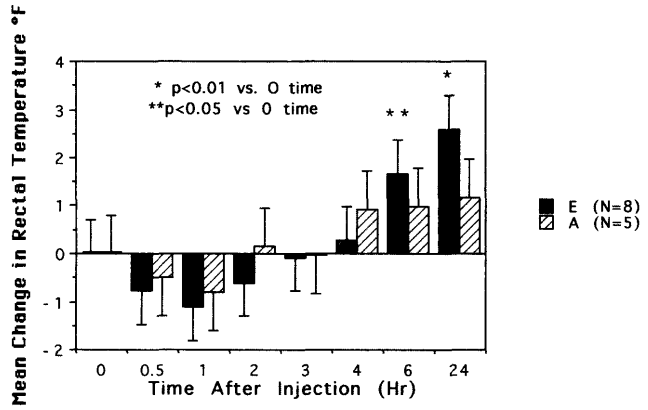

Figure 2. Effects of etiocholanolone and androsterone on rectal temperature in Rhesus monkeys. Each steroid was injected sc at a dose of 1.0 mg/kg at Time 0 (n is indicated in parantheses). Zero time temperatures (°F ± SD) were 100.0 ± 1.25 for E and 100.2 ± 0.78 for A. The error bars are the standard deviations. The rectal temperature of the E-injected monkeys differed significantly from the Time 0 temperature at 6 ($P < 0.05$) and 24 hr ($P < 0.001$).

< 0.001 vs. Time 0 and $P < 0.05$ vs A-injected Rhesus at 24 hr).

Intravenous injection of IL-1α (30ng/kg) resulted in significantly elevated rectal temperatures at 30 and 60 min in Squirrel monkeys and at 30, 60, and 120 min in Rhesus monkeys relative to controls injected with pyrogen-free 0.15 *M* NaCl vehicle (Fig. 4A and 4B; $P < 0.02$–0.005). The Squirrel monkey body temperature at 120 min was also significantly elevated relative to the initial rectal temperature following injection of IL-1α ($P = 0.005$).

Discussion

Injection of E at a dose of 1 mg/kg resulted in significant increases in rectal temperature in Rhesus monkeys over a 24-hr period. In contrast, doses up to 25 mg/kg [and 250-fold higher than those reported to induce fever in humans (4)] had no significant effect on rectal temperature in Squirrel monkeys. On the other hand, injections of small doses of IL-1α induced rapid increases in rectal temperature in both Rhesus and Squirrel monkeys.

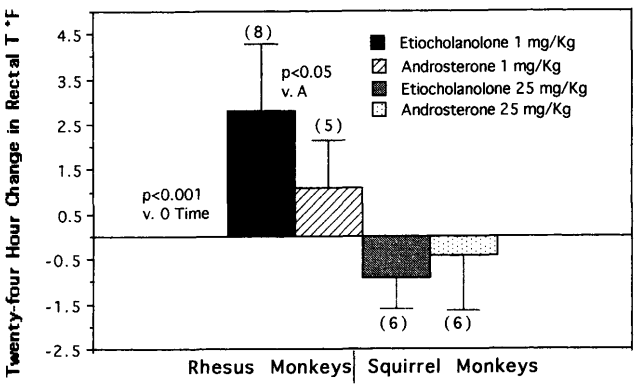

Figure 3. Summary of effects of E and A on rectal temperatures of Squirrel and Rhesus monkeys. The steroids were injected sc at Time 0. Only the change in rectal temperature of the Rhesus monkeys injected sc with 1 mg E differed significantly from initial body temperature ($P < 0.001$). This temperature change was also significantly greater than that of A-injected monkeys ($P < 0.05$).

The thermogenic effects of E are thought to result from accumulation of leucocytes at the injection site and the subsequent release of IL-1 (4, 5). The data therefore support the notion that the pyrogenic effects of E observed in Rhesus monkeys are the result of IL-1 release. In Squirrel monkeys, on the other hand, E injection exerted no discernable pyrogenic action, despite the clearcut demonstration that IL-1α, itself, was pyrogenic in this species. The logical conclusion to be derived from these events was that E injection did not result in release of IL-1 in Squirrel monkeys.

Two earlier reports on the thermogenic properties of steroids claimed that E and related steroids were species specific for humans, and not active as pyrogens in a variety of other mammals including Rhesus monkeys (15, 16). However, no data were given in either of these papers, and there was no reference to training the animals to acclimate them to injections and to insertion of rectal temperature probes. In the case of our own monkeys, a stable temperature baseline could not be achieved until the animals had been acclimated to these procedures by several weeks of training. Without such training it would have been impossible to distinguish between E-induced elevations in body temperature and those due to nonspecific stress. As an additional built-in control of the procedure itself, we routinely compared the effects of injections of E with those of its optical isomer, A, a nonpyrogenic steroid.

Squirrel monkeys are one of a group of New World Monkeys inhabiting South and Central America. During the course of evolution these animals have lost the ability to synthesize the liver 5α- and 5β-reductases that convert androgenic steroids to their eventual excretory products, E and A in Old World monkeys (including Rhesus monkeys), apes and humans (12, 13).

We conclude that E is pyrogenic in Rhesus monkeys, and the mechanism of this effect is likely similar to that

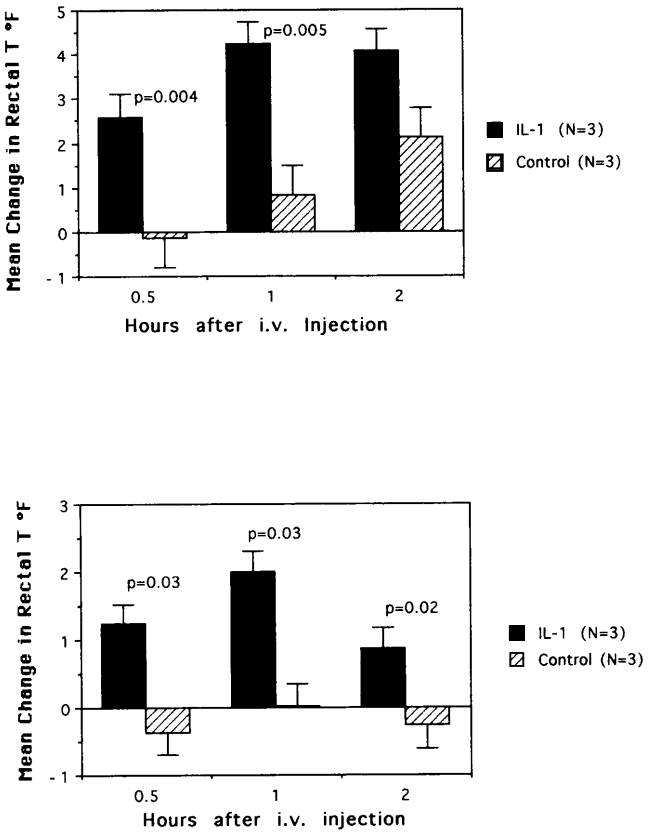

Figure 4. Effect of IL-1α on rectal temperature in Squirrel and Rhesus monkeys. The IL-1α or vehicle was injected iv at a dose of 30 ng/kg at Time 0. (A) Squirrel monkeys. Time 0 temperatures (°F ± SD) were 102.0 ± 0.71 for 3 IL-1α-treated and 101.8 ± 0.47 for 3 vehicle-treated monkeys. (B) Rhesus monkeys. Time 0 temperatures (°F ± SD) were 101.8 ± 0.21 for 3 IL-1α-treated and 102.8 ± 0.48 for 3 vehicle-treated controls. The bars represent the standard deviations. Rectal temperatures were significantly elevated at 30 and 60 min in Squirrel monkeys and 30, 60, and 120 min in Rhesus monkeys, relative to the saline-injected controls. The various *P* values are shown in the figure. Rectal temperatures of all monkeys were significantly elevated at each time interval relative to their pre-injection temperature ($P = 0.05$–0.005).

observed in humans (i.e., by release of the endogenous pyrogen, IL-1). E in doses up to 250 times the effective human dose did not elevate body temperature in Squirrel monkeys, and therefore did not release thermogenic quantities of IL-1 in this species. There are several possible explanations for this finding. Perhaps a metabolite of E rather than E itself releases IL-1, and Rhesus but not Squirrel monkeys are capable of converting E to such a hypothetical compound. Alternatively, in addition to losing the ability to synthesize E, New World Monkeys may also have lost the ability to "recognize" E as a biologically active steroid, such "recognition" possibly involving leukocyte receptors for E. These questions should be resolved by immunoassay of IL-1 plasma levels in Rhesus and Squirrel monkeys following injection of E, and by measuring IL-1 production by leukocytes from the two species following exposure to E *in vitro*.

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