Role of $\alpha$- and $\beta$-adrenoceptors in sympathetically mediated thermogenesis

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Blak, E. E., M. A. van Baak, K. P. G. Kempen, and W. H. M. Saris. Role of $\alpha$- and $\beta$-adrenoceptors in sympathetically mediated thermogenesis. Am. J. Physiol. 264 (Endocrinol. Metab. 27): E131-E136; 1993.—The study was intended to investigate the role of $\alpha$- and $\beta$-adrenoceptor populations in sympathetically mediated thermogenesis in healthy lean males. In the first study, the $\beta_1$, $\beta_2$, and $\beta_3$-agonist isoprenaline was infused in increasing doses with and without simultaneous infusion of the $\beta_1$-blocker atenolol (Iso and Iso + AT, respectively). There was an increase in whole body energy expenditure (EE) after infusing Iso + AT ($P < 0.001$) and an almost twofold higher increase after infusion of Iso only ($P < 0.001$). Stimulation of the $\beta_2$-adrenoceptors by a specific agonist (salbutamol) resulted in a significant increase in EE ($P < 0.001$). The effect of stimulation of $\alpha_1$-adrenoceptors on EE was measured by infusing increasing doses of the $\alpha_1$-agonist phenylephrine. EE did not change, whereas blood pressure (BP) increased ($P < 0.001$) and heart rate decreased ($P < 0.01$). In addition to this study, the $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, and $\beta_3$-agonists norepinephrine and epinephrine were infused with simultaneous infusion of the $\beta_1$- and $\beta_2$-blocker propranolol. In both studies, there was no effect on EE, whereas BP increased ($P < 0.01$). In conclusion, in healthy male lean volunteers both $\beta_1$- and $\beta_2$-adrenoceptors are involved in the sympathetically mediated thermogenesis, whereas the $\alpha_1$, $\alpha_2$, and $\beta_3$-adrenoceptors do not play a role. Alpha and beta adrenergic receptors; sympathetic; skeletal muscle

THE SYMPATHETIC NERVOUS system plays a role in the regulation of energy expenditure. Several studies have shown that there is an increase in energy expenditure as a result of infusion of norepinephrine or epinephrine (17, 29, 30). Also, there are indications that the facultative component of the diet-induced thermogenesis is mediated by an increased sympathetic tone (2, 27).

It is still uncertain which type of adrenoceptor mediates the sympathetically induced thermogenesis. Several studies have shown that the diet-induced thermogenesis can be inhibited by $\beta$-blockade (1, 4, 33). Thorin et al. (33) showed in a glucose-insulin clamp study that only $\beta_1$-adrenoceptors are involved in the sympathetically mediated thermogenesis, whereas Aastrup et al. (4) also suggested involvement of $\beta_2$-receptors. Additionally, atypical $\beta$-agonists (perhaps $\beta_3$) increased thermogenesis in rats (16) and in humans (14). In humans, a $\beta$-agonist with atypical selectivity increased weight loss in obese subjects on a restricted diet (8), although this effect is not seen in all studies (5). Furthermore, in a recent study (19), $\beta_3$-adrenoceptors could not be characterized in human white adipose tissue.

Less is known on the role of the $\alpha$-adrenergic receptors in the sympathetically mediated thermogenesis. In rats, norepinephrine-induced thermogenesis was markedly inhibited by $\alpha_1$-receptor blockade (13). However, in humans, $\alpha$-receptor blockade by phentolamine did not influence diet-induced thermogenesis (10, 28). So far, no studies have been done to investigate the effect of $\alpha$-adrenoceptor stimulation on resting energy expenditure.

The localization of the thermogenesis induced by the sympathetic nervous system is still a matter of dispute. Studies in adult rats and mice have shown that the major site for nonshivering or diet-induced thermogenesis is brown adipose tissue (31). However, Aastrup et al. (3) showed that, in humans, ephedrine-induced thermogenesis is not located in brown adipose tissue. They concluded that up to 50% of the increase in whole body oxygen consumption may take place in skeletal muscle.

This study was intended to investigate the role of $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, and $\beta_3$-adrenoceptors in the sympathetically mediated thermogenesis. The adrenoceptor population of skeletal muscle mainly consists of $\beta_2$-adrenoceptors (20). Therefore, it might be possible to obtain an indication of the different tissues contributing to the sympathetically mediated thermogenesis on the basis of the involved adrenoceptor populations.

SUBJECTS AND METHODS

General. The study design consisted of five protocols. In these studies the effects of several $\alpha$- and $\beta$-adrenoceptor agonists and antagonists on energy expenditure at rest were investigated, as summarized in Fig. 1. During all experiments, energy expenditure was continuously measured by an open-circuit ventilated hood system (Oxycon Beta; Jaeger, Brede, The Netherlands). The experiments were performed after a 12-h fast (overnight), and room temperature was kept between 23 and 25°C. Energy expenditure was calculated according to the abbreviated formula of Weir (35). The experiments started at 9:00 A.M. and the subjects came to the laboratory by car or bus. The study protocol was approved by the Ethics Committee of the University of Limburg, and all volunteers were asked for written consent. Before participation, all subjects underwent a medical examination.

Isoproterenol. In the first protocol 10 healthy male volunteers participated, aged 25.2 ± 1.7 (SE) yr and with a body mass index (BMI) of 22.5 ± 0.5 kg/m². The subjects were studied on two different occasions with 3-7 days in between. At the beginning of the experiment, catheters were inserted in a left and right forearm vein. At one experimental day, the $\beta_1$, $\beta_2$, and $\beta_3$-agonist isoprenaline (Iso) was infused in one arm and saline in the other arm. At the other experimental day, Iso was infused in one arm and the $\beta_1$-antagonist atenolol (AT) in the other arm. The study design was single-blind, and the order of treatments was randomized. After a 30-min baseline measurement, a continuous infusion of saline or AT (0.1 mg·kg body wt⁻¹·h⁻¹) was started. Before the infusion of AT was started, a priming dose of AT (0.07 mg/kg body wt) or saline was given within 5 min. After 60 min, in the other arm the infusion of Iso was started in doses of 5, 10, 20, and 40 ng·kg body wt⁻¹·min⁻¹, each dose for 30 min. The dose in the text is related to Iso sulfate, 69% of which corresponds to Iso-free base. During the experiment heart rate (HR) was recorded every 5 min. When HR had risen 30 beats/min or more, the infusion was stopped.

Salbutamol. In the second study, $\beta_2$-adrenoceptors were stimulated by the selective $\beta_2$-agonist salbutamol in seven healthy male volunteers (age 24.3 ± 1.8 yr, BMI 23.0 ± 0.6 kg/m²). At the beginning of the experiment a catheter was inserted in a forearm vein. After a 30-min baseline measurement, the $\beta_2$-agonist salbutamol was infused in increasing doses...
of 70, 140, and 280 ng·kg body wt\(^{-1}\)·min\(^{-1}\), each dose for 30 min. To exclude a possible \(\beta\)-effect, the highest dose of salbutamol was continued for 30 min in the presence of a continuous infusion of the \(\beta\)-blocker AT (bolus 0.07 mg/kg body wt, infusion 0.1 mg·kg body wt\(^{-1}\)·h\(^{-1}\)). During the experiments, HR was recorded every 5 min. When HR had risen 35 beats/min or more, the infusion was continued in the presence of AT.

**Phenylephrine.** The effect of stimulation of \(\alpha\)-receptors on resting energy expenditure was measured in six healthy male volunteers (age 24.0 ± 2.1 yr, BMI 21.7 ± 0.9 kg/m\(^2\)) by infusion of the \(\alpha\)-agonist phenylephrine. Before the experiment a catheter was inserted in a forearm vein for the infusion. After a 30-min baseline measurement, phenylephrine was infused in increasing doses of 0.5, 1, and 2 μg·kg body wt\(^{-1}\)·min\(^{-1}\), each dose for 30 min. During the experiments, HR and mean arterial pressure (MAP) were recorded every 5 min. The infusions were stopped when HR had decreased to 35 beats/min or when MAP had risen >30 mmHg.

**Norepinephrine and epinephrine plus propranolol.** In the last two protocols, the \(\alpha\)-, \(\alpha\)-, \(\beta\)-, \(\beta\)-, and \(\beta\)-agonists norepinephrine (NE, \(n = 6\), age 24.3 ± 2.1 yr, BMI 22.9 ± 0.6 kg/m\(^2\)) and epinephrine (Epi, \(n = 6\), age 24.5 ± 1.9 yr, BMI 22.7 ± 0.5 kg/m\(^2\)) were infused in the presence of \(\alpha\)- and \(\beta\)-blocker propranolol in healthy male volunteers. Before the experiments, two catheters were inserted in a left and right forearm vein for the infusion. After a 30-min control measurement, a priming injection of propranolol was given (195 μg/kg body wt), after which a continuous infusion of propranolol was started (0.6 μg·kg body wt\(^{-1}\)·min\(^{-1}\)). After 1 h, in addition, NE (0.025, 0.05, and 0.10 μg·kg body wt\(^{-1}\)·min\(^{-1}\)) or Epi (15, 30, 60, and 120 ng·kg body wt\(^{-1}\)·min\(^{-1}\)) was infused in the other arm (each dose for 30 min).

**Statistics.** Data are represented as means ± SE. For all protocols, statistical analysis was performed with repeated-measures analysis of variance. \(P < 0.05\) was regarded as statistically significant.

The total response to the infusion of Iso or Iaa and AT was calculated as the integrated area under the curve. For both treatments, the areas under the curve were compared by a paired \(t\) test.

**RESULTS**

**General.** During the infusion of increasing doses of Iso, salbutamol, phenylephrine, NE, or Epi, several subjects were not subjected to the higher infusion rates, due to our criteria for stopping the infusion (see SUBJECTS AND METHODS). In the first experiment, three subjects were not subjected to the last infusion period of Iso (40 ng·kg body wt\(^{-1}\)·min\(^{-1}\)). Furthermore, data on the highest infusion rates in protocols two to five are not presented because these infusion rates were not started in most subjects.

After 10 min of infusion of Iso, Iso plus AT, phenylephrine, NE, or Epi (both with propranolol), energy expenditure and HR had reached a steady state, i.e., 5-min values did not significantly change anymore until the end of the infusion period. Therefore, mean values over the last 20 min of infusion were taken as representative for the administered dose. In the Iso and Iso plus AT trial, steady-state values for the respiratory exchange ratio (R)

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*Fig. 1. Study design of 5 protocols. Iso, isoprenaline; NE, norepinephrine; E, epinephrine; REE, resting energy expenditure; n, no. of subjects. a Atenolol was infused with highest dose of salbutamol (140 or 280 ng·kg\(^{-1}\)·min\(^{-1}\)).*
over 5-min intervals were achieved after 20 min of infusion. For this reason, R values of the last 10 min of infusion were averaged. With infusion of NE and Epi (both with propranolol) and with phenylephrine, R values were stable throughout the infusions. With infusion of the β₂-agonist salbutamol, energy expenditure, HR, and R reached a steady state within 20 min.

Iso. There was a significant increase in resting energy expenditure as a result of the infusion of the β₁-, β₂-, and β₂-agonist Iso. For the different doses, this increase was 7% at 5 ng·kg body wt⁻¹·min⁻¹, 13% at 10 ng·kg body wt⁻¹·min⁻¹, 16% at 20 ng·kg body wt⁻¹·min⁻¹, and 25% at 40 ng·kg body wt⁻¹·min⁻¹. As a result of the simultaneous infusion of Iso and the β₁-blocker AT, there also was a significant increase in resting energy expenditure (compared with infusion of AT only), which was one-half the increase with infusion of Iso only (for the increasing doses, respectively, 8, 6, 10, 14%; Fig. 2). The integrated increase in resting energy expenditure was significantly higher with infusion of Iso than with Iso plus AT (P < 0.001). Infusion of the β₁-blocker atenolol for 30 min did not affect resting energy expenditure (control 5.14 ± 0.12 vs. AT 5.06 ± 0.10 kJ/min).

The preinfusion R was 0.83 ± 0.014 in the Iso trial and 0.85 ± 0.010 in the Iso plus AT trial. With Iso, there was a marked increase in R within the first 5 min of infusion, most probably caused by a change in ventilation, which decreased again within the next 10 min. Steady-state R values (the last 10 min of infusion) significantly decreased with infusion of increasing doses of Iso (P < 0.01), whereas with Iso plus AT there was no change in R. HR significantly increased with both treatments (P < 0.001), and the increase with Iso plus AT was one-half of the increase with Iso only. The integrated HR response was significantly higher with Iso than with Iso plus AT (P < 0.001), which indicates considerable β₁-blockade.

Salbutamol. Stimulation of the β₂-adrenoceptors by salbutamol increased energy expenditure by 14% at 70 ng·kg body wt⁻¹·min⁻¹ and by 19% at 140 ng·kg body wt⁻¹·min⁻¹ (Fig. 3). Simultaneous infusion of the highest dose of salbutamol and the β₁-blocker AT resulted in a 2%
lower energy expenditure than with infusion of salbutamol only (6.66 ± 0.39 vs. 6.54 ± 0.35 kJ/min, not significant), whereas HR significantly decreased (92.0 ± 5.8 vs. 81.2 ± 3.2 beats/min). Preinfusion R was 0.85 ± 0.018, and steady-state values did not change as a result of the infusion. There was a significant increase in HR as a result of the β2-adrenergic stimulation (for the 2 doses, respectively, 11 and 27 beats/min, P < 0.001, Fig. 3).

Phenylephrine. Infusion of increasing doses of the α1-agonist phenylephrine did not change basal energy expenditure (Fig. 4), whereas there was a significant decrease in HR (P < 0.01) and a significant increase in MAP (P < 0.001). The basal R was 0.84 ± 0.016, and this value did not change as a result of the infusion of phenylephrine.

**NE and Epi plus propranolol.** As result of the infusion of the β1- and β2-blocker propranolol, there was a very small significant decrease in resting energy expenditure (n = 12, 5.35 ± 0.21 vs. 5.20 ± 0.20 kJ/min, P < 0.01), whereas R did not change significantly. Simultaneous infusion of the α1-, α2-, β1-, β2-, and β3-agonists NE or Epi and the β1- and β2-blocker propranolol did not change energy expenditure (compared with propranolol, Fig. 5). R did not significantly change as a result of the infusion of NE or Epi and propranolol (preinfusion value 0.83–0.85). With both infusions, there was an increase in MAP (P < 0.001) and a decrease in HR (NE P < 0.01 and Epi P < 0.001). Table 1 summarizes the results of the above mentioned protocols with respect to energy expenditure.

**DISCUSSION**

The objective of the present study was to investigate the role of the different adrenoceptor populations in the sympathetically mediated thermogenesis. In the literature, it has been reported that β1- and β2-adrenoceptors may be involved in the adrenergically mediated thermogenesis (4, 33), whereas the role of the atypical β3-adrenoceptors remains controversial (6, 8). Less is known on the role of the α-adrenoceptors in the sympathetically mediated thermogenesis.

In the present study, there was a significant increase in resting energy expenditure after simultaneous infusion of the β1-, β2-, and β3-agonist Iso in combination with the β1-blocker AT and a twofold higher increase with infusion of Iso only. An increase in energy expenditure as a result of infusing Iso has previously been reported. Cobbald et al. (7) showed an increase in energy expenditure with an infusion of 100 ng·kg body wt⁻¹·min⁻¹. Mansell et al. (22) reported increases in HR and energy expenditure with a 5- and 15-ng·kg body wt⁻¹·min⁻¹ infusion that seem to be slightly higher than our values. The increase in HR with Iso infusion in our study is comparable to data of Martinsson et al. (23). The administered dose of the β1-blocking agent AT was similar to those previously shown to be sufficient for an effective β1-blockade (9, 34) and for a high β1-selectivity (21). That there was a large β1-adrenergic blockade was evident from the finding that HR increase was halved after β1-blockade. From literature, it is known that Iso-induced tachycardia decreases to the same degree after β1- or β2-blockade, which indicates that both β1- and β2-adrenoceptors contribute equally to this response (24). The involvement of the β2-adrenoceptors in thermogenesis is confirmed in the second study, in which there was a significant rise in energy expenditure with infusion of the β2-agonist salbutamol. Simultaneous infusion of the highest dose of salbutamol and the β1-blocker AT resulted in a nonsignificant 2% lower energy expenditure than with infusion of salbutamol only, which shows that most of the rise in energy expenditure was due to β2-adrenergic stimulation. From these findings, we can conclude that both β1- and β2-adrenoceptors are involved in the sympathetically mediated thermogenesis.

Stimulation of α1-adrenoceptors did not change energy expenditure, which shows that these adrenoceptors are
ADRENOCEPTORS AND THERMOGENESIS

Fig. 5. Changes in energy expenditure (EE), mean arterial blood pressure (MAP), and heart rate (HR) as result of infusion of $\alpha_1$-, $\alpha_2$-, $\beta_1$-, $\beta_2$-, and $\beta_3$-agonists norepinephrine (NE, left) and epinephrine (Epi, right) with simultaneous infusion of $\beta_1$- and $\beta_2$-blocker propranolol (means ± SE, n = 6). **P < 0.01 and ***P < 0.001 by repeated-measurements ANOVA.

not involved in the adrenergically mediated thermogenesis. That there was a significant stimulation of $\alpha_1$-adrenoceptors is evident from the increase in MAP during the infusion periods. The role of the $\alpha_2$-adrenoceptors was determined by infusing NE or Epi in the presence of the $\beta_1$- and $\beta_2$-blocker propranolol. There was no change in energy expenditure and a significant increase in MAP, which shows that $\alpha_2$-adrenoceptors are not (directly) involved in thermogenesis. We cannot exclude the possibility that $\alpha_2$-adrenoceptors have indirect effects on thermogenesis by modulating $\beta$-adrenergic effects on metabolic processes (18). However, these effects are probably of the same magnitude as the effects of $\alpha_1$-adrenoceptor stimulation on thermogenesis and therefore are very small. The above results are comparable to data previously reported in humans, where $\alpha$-adrenoceptor blockade did not influence diet-induced thermogenesis (10, 28).

Reports on the involvement of $\beta_3$-adrenoceptors in thermogenesis in humans are controversial (5, 8, 19). Both increased (8) and unchanged (5) weight losses have been reported as a result of adding a $\beta_3$-agonist to a restricted diet in obese subjects. In our study, stimulation of the nonclassical $\beta_3$-adrenoceptors by infusing NE and Epi in the presence of the $\beta_1$- and $\beta_2$-blocker propranolol did not change energy expenditure. It has been reported that NE appears to be the best endogenous agonist of
Table 1. Changes in EE as a result of infusion of several α- and/or β-adrenoceptor agonist or antagonists

<table>
<thead>
<tr>
<th>Protocol</th>
<th>EE</th>
<th>%Change in EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Isoprenaline (β₁ and β₂)</td>
<td>Increase</td>
<td>25% at 40 ng·kg body wt⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Isoprenaline with atenolol (β₂)</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Atenolol (β₁-blockade)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Salbutamol (β₂)</td>
<td>Increase</td>
<td>14% at 40 ng·kg body wt⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Salbutamol with atenolol (β₂)</td>
<td>Increase</td>
<td>19% at 140 ng·kg body wt⁻¹·min⁻¹</td>
</tr>
<tr>
<td>3) Phenylephrine (α₁)</td>
<td>No change</td>
<td>2% lower than increase with infusion of highest dose salbutamol only</td>
</tr>
<tr>
<td>4) Norepinephrine with propranolol (α and β₁)</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Epinephrine with propranolol (α and β₁)</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Propranolol (β₁- and β₂-blockade)</td>
<td>Decrease</td>
<td>3% decrease in resting EE</td>
</tr>
</tbody>
</table>

EE, energy expenditure.

β₂-adrenoceptors (11). The activation constant (Kₘ) for adenosine 3',5'-cyclic monophosphate accumulation in eukaryotic cells (Chinese hamster ovary cells) transfected with a human β₂ gene is 6.3 ± 0.7 nM/l for NE, whereas the sensitivity of Epi is somewhat lower (Kₘ 49.2 ± 5.3 nM/l). The standard β₁- and β₂-blocker propranolol had only very limited effects on the human β₂-adrenergic receptor (11). Hjedahl et al. (15) reported that β₂-adrenoceptor blockade (by propranolol) increases the plasma concentrations of NE and Epi during NE or Epi infusion. With infusion, rates of NE and Epi (and propranolol) comparable to our highest infusion rates plasma concentrations above the "physiological" range are achieved (NE > 10 nM, Epi > 4 nM). From the above considerations, we can conclude that, in healthy lean males, physiological concentrations of circulating catecholamines, NE and Epi have no effect on thermogenesis mediated by β₂-adrenoceptors. However, we cannot completely exclude involvement of β₁-adrenoceptors in situations with a high local release of NE. From the literature, it is known that Iso also displays very strong β₁-adrenergic agonist activity (Kₘ 3.9 ± 0.4 nM; see Ref. 10). An effect of Iso on thermogenesis via the β₂-adrenoceptor seems very unlikely, because even very high concentrations of the catecholamines are not able to stimulate β₂-mediated thermogenesis. Thus, since in healthy lean males the β₂-adrenoceptors probably do not play a role in the Iso-induced increase in energy expenditure and since the Iso-induced increase in energy expenditure is halved as a result of β₁-blockade, the contribution of the β₁- and β₂-adrenoceptors to thermogenesis seems to be of similar magnitude.

As a result of β₁- and β₂-adrenergic blockade by propranolol, there was a very small (3%) significant decrease in resting energy expenditure. This indicates the involvement of the β₂-adrenergic system in resting energy expenditure. In literature, both unchanged (37) or decreased (36) resting energy expenditures have been reported as a result of β₂-adrenergic blockade. This discrepancy may be due to methodological difficulties in detecting such a small difference. Additionally, Christin et al. (6) reported after propranolol infusion, a negative correlation between the decrease in resting energy expenditure and the abdomen-to-thigh ratio, which suggests that differences in study population may also contribute to the inconsistent results in the literature.

Astrup et al. (3) showed that, in humans, ephedrine-induced thermogenesis is not located in brown adipose tissue as in rats, but suggested that up to 50% of the increase in whole body oxygen consumption may take place in skeletal muscle. The quantitative significance of skeletal muscle and other tissues in the adrenergically mediated thermogenesis cannot be established from the present study. However, the fact that β₂-adrenoceptors are involved in the sympathetically mediated thermogenesis makes it very likely that skeletal muscle is an important site of the localization because the adrenoceptor population of skeletal muscle mainly consists of β₂-adrenoceptors (20). Additionally, skeletal muscle accounts for 40–50% of body weight in normal-weight volunteers (25) and is therefore, quantitatively, the most important tissue mass of the body. The involvement of β₁-adrenoceptors shows that other tissue, such as adipose tissue, must also play a role. The energy cost of the elevated cardiac and respiratory work as a result of sympathetic stimulation is generally considered to account for only a low percentage of its stimulatory effect on metabolic rate (29).

The present study offers no explanation for the processes that cause the sympathetically mediated energy expenditure. The decrease in steady-state values of R with increasing doses of Iso, which seems to be due to metabolic effects of Iso (12), suggests an increased rate of lipolysis. Furthermore, adrenergic stimulation has been reported to result in a fall in plasma K⁺ concentrations (3, 26). This, together with the sympathetically induced increase in oxygen consumption (3), suggests stimulation of active Na⁺-K⁺ pump activity takes place predominantly via β₂-adrenoceptors, but β₁-adrenoceptors may also play a part (26). Futility cycles have also been reported to be responsible for a part of the sympathetically mediated thermogenesis (32). These cycles could have a major role in increasing the sensitivity of processes that control the rates of degradation and synthesis of fuel stores.

In conclusion, in healthy lean males β₁- and β₂-adrenoceptors are involved in the sympathetically mediated thermogenesis, whereas the α₁, α₂- and β₁-adrenoceptors are not. The involvement of β₂-adrenoceptors makes it very likely that part of the sympathetically mediated increase in thermogenesis takes place in skeletal muscle, but other tissues containing β₁-adrenoceptors must also be involved.

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