

## Prostaglandin-related Microvascular Dilation in Pentobarbital- and Etomidate-anesthetized Rats

Eleanor F. Asher, M.D.,\* Nancy L. Alsip, Ph.D.,† Ping Y. Zhang, M.D.,‡ Patrick D. Harris, Ph.D.,§

Etomidate is characterized by minimal systemic cardiovascular effects, but its effect on the microvasculature has not been assessed. We compared the microvasculature of etomidate-anesthetized animals to that of animals anesthetized with pentobarbital, since its effects on the microvasculature are known. Male Sprague-Dawley rats were anesthetized with etomidate or pentobarbital. The cremaster muscle was prepared for microscopic viewing, leaving the neural and vascular supply intact. Small arterioles were near their maximal diameters in etomidate-anesthetized rats, whereas the pentobarbital group had a large dilator capacity (maximal diameter - basal diameter/basal diameter). The effect on resting arteriolar diameters of endothelium-derived relaxing factor (EDRF) and prostaglandin synthesis inhibitors was tested. Dilator capacity was not affected by the EDRF inhibitor nitro-L-arginine, but it was significantly increased by mefenamic acid and ibuprofen in etomidate-anesthetized animals. To test whether dilator and constrictor mechanisms were normal, serotonin concentration-response curves were obtained in pentobarbital and etomidate-anesthetized animals with and without mefenamate or ibuprofen present. The dilation of small arterioles to serotonin in the etomidate groups with mefenamate or ibuprofen was not significantly different from that of the pentobarbital groups. Serotonin produced a comparable constriction of large arterioles in both anesthetic groups. The topical application of etomidate to the cremaster muscle did not affect arteriolar diameters. Thus, etomidate appears to trigger the release of dilator prostaglandins in striated muscle through a central or indirect mechanism. (Key words: Anesthetics, intravenous: etomidate, pentobarbital. Hormones: Prostaglandins. Microcirculation. Muscle, smooth: vascular.)

ETOMIDATE, A CARBOXYLATED IMIDAZOLE, intravenous hypnotic anesthetic agent, is characterized by dose-related cardiovascular or respiratory effects,<sup>1-11</sup> no histamine release,<sup>12</sup> and a high therapeutic index (six times greater than that of thiopental).<sup>13</sup> No assessment has been made of the effects of this agent on the microvasculature.

The primary purpose of this study was to determine the effect of etomidate on baseline arteriolar tone in skeletal muscle. Our second goal was to assess whether the response of the microvasculature to serotonin (5-hy-

droxytryptamine) was altered in etomidate-anesthetized rats. Serotonin is unique in that it acts as both a vasodilator and as a vasoconstrictor at different arteriolar levels in the cremaster<sup>14</sup> as well as other microcirculatory beds.<sup>15,16</sup> In this study serotonin was used as a test agonist to explore whether etomidate alters vasoconstrictor or vasodilator mechanisms or the sensitivity of the microvessels to serotonin.

In preliminary studies using etomidate-anesthetized animals, we found that the small precapillary arterioles were maximally dilated; *i.e.*, they had little or no basal arteriolar tone. Our third goal was to determine the etiology of this dilation. Prostaglandins and endothelium-derived relaxing factor (EDRF) are well-known vasodilators and have a major role in vascular control.<sup>17-19</sup> We therefore chose to evaluate the possible involvement of prostaglandins and EDRF in the etomidate-induced dilation of small arterioles.

We compared the etomidate-anesthetized animals to animals anesthetized with pentobarbital as it is frequently used in animal studies and its effects on the microcirculation are known.<sup>20</sup>

### Materials and Methods

Male Sprague-Dawley rats were used for all experiments. These animals arrived as weanlings (50-75 g) and were caged individually in a room that was temperature- and humidity-controlled with a 12-h light/12-h dark cycle. They were fed standard laboratory rat chow with access to tap water *ad libitum* until body weight reached 140-160 g (approximately 14-18 days postweanling, or 5-6 weeks of age). Protocols were approved by the University of Louisville Institutional Animal Care and Use Committee.

On each day of an acute experiment, a rat was anesthetized with either etomidate or sodium pentobarbital as described below. Two experiments were performed per day. The anesthetic agent used was randomized so that half of each group was anesthetized in the morning and half in the afternoon. No effect due to the difference in the time of day was detected.

### ANESTHETICS

Etomidate was given in an initial dose of 20 mg/kg intraperitoneally. After placing a venous catheter, etomidate was infused at a rate of  $7.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  intrave-

\* Assistant Professor.

† Senior Research Associate, Department of Physiology.

‡ Research Associate, Department of Surgery.

§ Professor, Department of Physiology.

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Address reprint requests to Dr. Asher: Department of Anesthesiology, School of Medicine, University of Louisville, Louisville, Kentucky 40292.

nously. Pentobarbital was administered in an initial dose 50 mg/kg intraperitoneally. Supplemental doses (16 mg/kg) were given subcutaneously every hour or infused at a rate of  $16 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  intravenously. The rats receiving maintenance doses of pentobarbital subcutaneously received intravenous saline at the same infusion rate ( $3.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) as etomidate or pentobarbital in the other groups. The route of maintenance pentobarbital administration did not affect the physiologic parameters or the microvascular responses. The rats appeared to be at a surgical level of anesthesia. Arterial blood pressure, heart rate, and respiratory rate did not fluctuate, and the animals did not respond to painful stimuli. Any animal that had large fluctuations in arterial pressure or heart rate, or in which arterial pressure diminished to a mean of less than 80 mmHg, was excluded from the study.

#### CREMASTER PREPARATION

In etomidate- or pentobarbital-anesthetized rats, one carotid artery and the trachea were cannulated in order to monitor arterial pressure and to maintain a patent airway; a jugular cannula was placed to infuse etomidate, pentobarbital, or saline.

The rectal temperature of the animals was monitored and maintained at  $37\text{--}38^\circ \text{C}$  by a back heating pad. The arterial pressure and heart rate of the animals was continuously monitored and recorded. Blood gas measurements were taken for one etomidate and one pentobarbital anesthetized group (etomidate-ibuprofen [ETO-IBU] and pentobarbital-ibuprofen [PB-IBU]). Arterial blood (0.4 ml) was taken approximately 1.5 h after the induction of anesthesia and was measured on a Ciba-Corning model 170 Blood-Gas Analyzer for  $\text{PaO}_2$ ,  $\text{PaCO}_2$ , and  $\text{pH}$ .

To prepare the cremaster muscle for microscopic observation, the right scrotal sac was incised, and the cremaster-enclosed testicle was gently dissected free of connective tissue. The cremaster muscle was then incised and dissected free of the testicle. This procedure left intact circulatory and neural connections to the cremaster.

The testicle was gently pushed into the abdominal cavity. The animal was placed on a Plexiglas board, and the cremaster muscle positioned over a cover glass, which served as an optical port, in the bottom of a 70-ml tissue bath that was attached to the Plexiglas board. The cremaster muscle was secured with sutures so that it was nearly flat over the optical port.

The entrance to the bath was sealed with a dam of grease and Parafilm, and the bath was filled with a modified Krebs solution (25.5 mM  $\text{NaHCO}_3$ , 112.9 mM  $\text{NaCl}$ , 4.7 mM  $\text{KCl}$ , 2.55 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 1.19 mM  $\text{KH}_2\text{PO}_4$ , 1.1 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 11.6 mM dextrose). The entire board was positioned on a microscope stage. Bath temperature was maintained at  $34.5 \pm 0.5^\circ \text{C}$  by an in-

dwelling bath heating unit electronically controlled *via* a temperature probe positioned in the bath.  $\text{N}_2$  and  $\text{CO}_2$  gases were bubbled continuously through the cremaster bath to serve three purposes: 1) the gas bubbling constantly stirred the bath; 2) the  $\text{CO}_2$  flow provided  $\text{pH}$  control; and 3) the  $\text{N}_2$  flow rate regulated bath  $\text{PO}_2$  by carrying away dissolved  $\text{O}_2$ . These gas flows were set to maintain bath  $\text{pH}$  at  $7.4 \pm 0.05$ ,  $\text{PO}_2$  at  $40 \pm 10$  mmHg, and  $\text{PCO}_2$  at 35–40 mmHg.

The microvessels in the cremaster were viewed at approximately  $850\times$  magnification on a monitor that was part of a closed-circuit television microscopy system. Vessel diameters were measured directly off the monitor screen.

#### MICROVASCULAR DATA

Following all surgical procedures in the anesthetized animals, there was a stabilization period of 30–60 min. During that time, all physiologic parameters were monitored for stable values and microvessels were selected for observation. In all experiments, the diameters of the major large arteriole (A1) that supplies the cremaster were measured. In addition, smaller third-order arterioles (A3) also were observed. Only A3 vessels with spontaneous vasomotion and those in the same general area of the cremaster muscle for each animal were selected. The data from one arteriole per animal were reported. Therefore, a group for which  $n = 6$  refers to six separate animals.

Since the experimental protocol called for 1-min of vessel diameters and vasomotion, the microvascular images were recorded on videotape to be read after completion of the experimental procedures.

#### PROTOCOLS

Following the stabilization period, there was a 15-min baseline period. At the beginning of the baseline period, one of three solutions was added to the cremaster bath. The two control groups (etomidate-control [ETO-CON] and pentobarbital-control [PB-CON]) received 0.01 M  $\text{Na}_2\text{CO}_3$  (the mefenamic acid vehicle). In two groups (etomidate-mefenamic acid [ETO-MEF] and pentobarbital-mefenamic acid [PB-MEF]), the cyclooxygenase inhibitor mefenamic acid (mefenamate) was added to the cremaster bath in a concentration of  $4 \times 10^{-5}$  M. The last two groups (ETO-IBU and PB-IBU) received ibuprofen (another cyclooxygenase inhibitor) at a bath concentration of  $9.6 \times 10^{-5}$  M. Basal diameters were established by measuring vessel diameters and frequency of vasomotion at 1-min intervals during the baseline period. Serotonin then was added directly to the cremaster bath to give a final bath serotonin concentration of  $1 \times 10^{-9}$  M. This and all subsequent concentrations of serotonin remained in the bath for 10 min. The concentration of se-

rotonin was increased in 10-fold increments (for 10 min each) until the last serotonin concentration in the bath was  $1 \times 10^{-4}$  M. Vessel diameters and vasomotion were measured at 1-min intervals during each 10-min serotonin period. At the end of the last serotonin period, the cremaster bath was drained and refilled five times with fresh Krebs solution over the next 20 min to wash out the serotonin in the bath. Sodium nitroprusside ( $10^{-5}$  M) was added to the bath to achieve the maximally relaxed diameter.

In a second protocol, two groups (etomidate-nitro-L-arginine [ETO-NOLA] and pentobarbital-nitro-L-arginine [PB-NOLA]) received the EDRF synthesis inhibitor nitro-L-arginine<sup>21,22</sup> at the beginning of the baseline period at a bath concentration of  $2 \times 10^{-4}$  M. After 10 min, vessel diameters were measured every 1 min for 10 min. The bath was then drained and refilled with fresh Krebs solution, and the EDRF-independent vasodilator sodium nitroprusside ( $10^{-5}$  M) was applied to the cremaster to achieve the maximally relaxed diameter.

In a third protocol, the animals (pentobarbital-etomidate [PB-ETO]) were anesthetized with pentobarbital as described above (intravenous infusion). After the stabilization period and a 30-min baseline period, etomidate (28  $\mu$ g/ml) was added to the cremaster bath (topical administration). A1 and A3 arteriolar diameters were measured every 5 min for 40 min. After the etomidate was washed out of the bath, sodium nitroprusside was applied as described above.

#### DATA ANALYSES

Statistical comparisons were done by application of an unpaired *t* test when comparing two groups (for the physiologic parameters). To identify differences among more than two animal groups, a one- or two-way analysis of variance followed by Bonferroni's method for multiple comparisons was applied. Differences were considered significant at the  $P < 0.05$  level. The dilator capacity was determined by subtracting basal (baseline) diameter from the maximally relaxed diameter and then dividing by the basal diameter. This number was multiplied by 100 in order to express it as a percentage. All data are reported as the means  $\pm$  SEM.

#### REAGENTS

Etomidate (Amidate, Abbott Laboratories, North Chicago, IL) was dissolved in propylene glycol (35% volume/

TABLE 1.  $P_{aO_2}$ ,  $P_{aCO_2}$ , and pH in Etomidate- and Pentobarbital-anesthetized Rats

	$P_{aO_2}$	$P_{aCO_2}$	pH
Etomidate (n = 5)	$88 \pm 4$	$33 \pm 2$	$7.42 \pm 0.01$
Pentobarbital (n = 5)	$77 \pm 5$	$37 \pm 2$	$7.43 \pm 0.02$

TABLE 2. Mean Arterial Pressure and Heart Rate in Etomidate- and Pentobarbital-anesthetized Rats

	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)
Etomidate (n = 28)	$114 \pm 3$	$403 \pm 22$
Pentobarbital (n = 29)	$109 \pm 5$	$383 \pm 17$

volume in saline). Pentobarbital (Na salt, Sigma Chemical, St. Louis, MO), serotonin (5-hydroxytryptamine HCl, Sigma), nitroprusside (Na nitroferricyanide, Sigma), and ibuprofen (Motrin, a gift from the UpJohn Company, Kalamazoo, MI) were made up in normal saline. Mefenamic acid (Sigma) was dissolved in 0.1 M  $Na_2CO_3$ .  $N\omega$ -nitro-L-arginine (Sigma) was dissolved in normal saline by stirring at room temperature. The chemicals for the Krebs solution were dissolved in distilled water and were all obtained from Sigma; they included the following:  $NaHCO_3$ , NaCl, KCl,  $CaCl_2 \cdot 2H_2O$ ,  $KH_2PO_4$ ,  $MgSO_4 \cdot 7H_2O$ , and dextrose. All reagents were made daily except the etomidate and ibuprofen, which came in solution from the suppliers.

#### Results

Arterial blood gas measurements were taken 1.5 h after the induction of anesthesia in ten animals (five with each anesthetic). There were no differences between the  $P_{aO_2}$ ,  $P_{aCO_2}$ , or pH of the etomidate- and pentobarbital-anesthetized rats (table 1). Mean arterial pressures and heart rates were pooled for all animals anesthetized with each anesthetic (table 2). There were no differences in mean arterial pressure or heart rate between the pentobarbital- and etomidate-anesthetized rats, suggesting that the depth of anesthesia and sympathetic activation were similar in all animals.

#### A3 ARTERIOLAR TONE

In etomidate-anesthetized rats (ETO-CON), the A3 arterioles had very little dilator capacity; *i.e.*, they possessed little basal tone. The ETO-CON group had a dilator capacity of only  $22 \pm 7\%$  compared to  $119 \pm 15\%$  for the PB-CON group (table 3). The dilator capacity was not increased by inhibiting EDRF synthesis with nitro-L-arginine in either the etomidate- (table 3, ETO-NOLA) or pentobarbital- (table 3, PB-NOLA) anesthetized groups.

In the presence of mefenamic acid, the dilator capacity of the A3 arterioles in the etomidate-anesthetized animals (table 3, ETO-MEF) was increased to  $156 \pm 38\%$ ; *i.e.*, the vessels constricted in the presence of mefenamate. In the pentobarbital-anesthetized (table 3, PB-CON) animals, mefenamic acid did not significantly increase the dilator capacity ( $122 \pm 25\%$ ). The dilator capacity of the vessels

TABLE 3. Dilator Capacity of Third-order Arterioles in Etomidate- and Pentobarbital-anesthetized Rats

Group	Basal Diameter ( $\mu\text{m}$ )	Maximal Diameter ( $\mu\text{m}$ )	Dilator Capacity* (%)
ETO-CON (n = 11)	22 $\pm$ 2†	27 $\pm$ 3	22 $\pm$ 7†
ETO-NOLA (n = 5)	33 $\pm$ 4†	37 $\pm$ 4	18 $\pm$ 11†
ETO-MEF (n = 6)	12 $\pm$ 3	28 $\pm$ 4	156 $\pm$ 38
ETO-IBU (n = 6)	16 $\pm$ 3	31 $\pm$ 4	97 $\pm$ 16
PB-CON (n = 11)	13 $\pm$ 2	28 $\pm$ 2	119 $\pm$ 15
PB-NOLA (n = 7)	13 $\pm$ 2	26 $\pm$ 6	98 $\pm$ 18
PB-MEF (n = 6)	11 $\pm$ 1	23 $\pm$ 1	122 $\pm$ 22
PB-IBU (n = 5)	11 $\pm$ 1	22 $\pm$ 4	120 $\pm$ 36

ETO = etomidate; CON = control; NOLA = nitro-L-arginine; MEF = mefenamic acid; IBU = ibuprofen; PB = pentobarbital.

\* Dilator capacity = (maximal-basal diameter)/basal diameter  $\times$  100.  
† Significantly different from other ETO groups and corresponding PB groups by two-way ANOVA ( $P < 0.05$ ).

exposed to ibuprofen was increased to  $97 \pm 16\%$  in the etomidate-anesthetized rats (table 3, ETO-IBU). This was significantly larger than the dilator capacity of the ETO-CON group. Ibuprofen did not significantly alter the dilator capacity of the pentobarbital-anesthetized rats (table 3, PB-IBU).

### A3 RESPONSE TO SEROTONIN

The response of the A3 arterioles to serotonin was tested in the control (ETO-CON and PB-CON), mefenamic acid-treated (ETO-MEF and PB-MEF), and ibuprofen-treated (ETO-IBU and PB-IBU) groups. Serotonin produced a concentration-dependent dilation of A3 arterioles (fig. 1, solid bars) in the PB-CON group. The A3 arterioles in the ETO-CON group (fig. 1, open bars) did

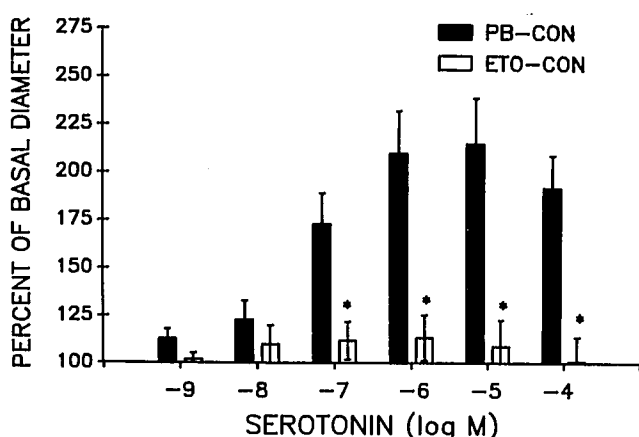


FIG. 1. The concentration-response curves to serotonin in A3 arterioles in the pentobarbital-control (n = 11) and etomidate-control (n = 11) groups. The ordinate gives the data as the percent of the basal diameters (table 3), where values greater than 100 indicate dilation. \*Significantly different from all other group responses,  $P < 0.05$ .

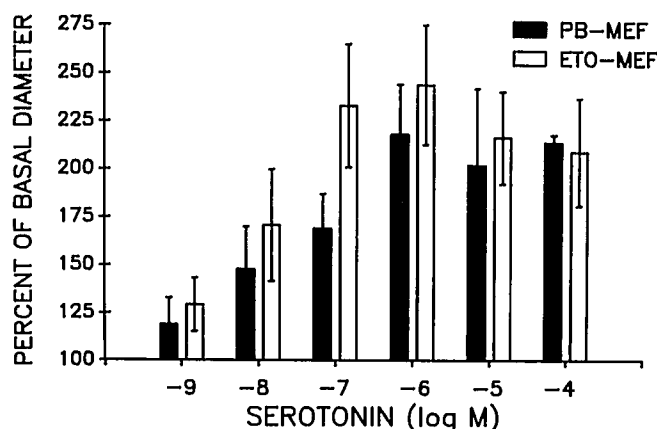


FIG. 2. The concentration-response curves to serotonin in A3 arterioles in the pentobarbital-mefenamic acid (n = 6) and etomidate-mefenamic acid (n = 6) groups during cyclooxygenase blockade with mefenamic acid. The ordinate gives the data as the percent of the basal diameters (table 3), where values greater than 100 indicate dilation.

not dilate to serotonin because these vessels were already near their maximal diameters (table 3). The dilation induced by serotonin was not altered by blocking cyclooxygenase with mefenamic acid in the PB-MEF group (fig. 2, solid bars), but in the ETO-MEF group (fig. 2, open bars) serotonin now gave a concentration-dependent dilation that was not different from the response of the PB-MEF or PB-CON groups. The same pattern was found for those groups in which cyclooxygenase was blocked with ibuprofen; i.e., serotonin dilated both the PB-IBU and ETO-IBU groups to a similar degree (fig. 3), and this response was not different from the response of the PB-CON, PB-MEF, or ETO-MEF groups.

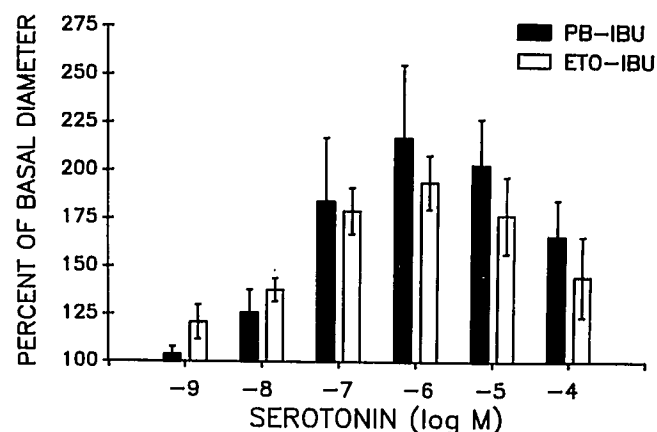


FIG. 3. The concentration-response curves to serotonin in A3 arterioles in the pentobarbital-ibuprofen (n = 5) and etomidate-ibuprofen (n = 6) groups during cyclooxygenase blockade with ibuprofen. The ordinate gives the data as the percent of the basal diameters (table 3), where values greater than 100 indicate dilation.

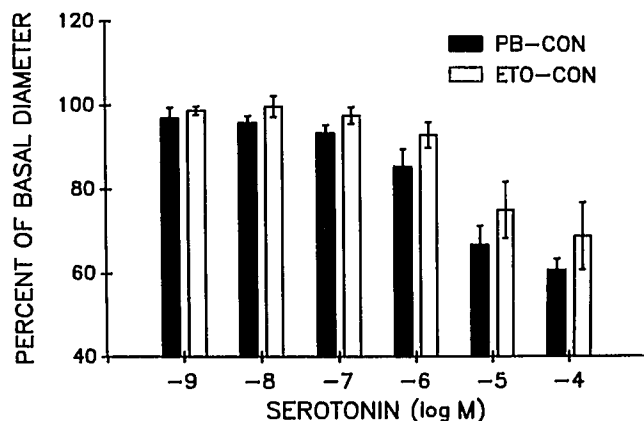


FIG. 4. The concentration-response curves to serotonin in large distributing arterioles (A1) in the pentobarbital-control ( $n = 11$ ) and etomidate-control ( $n = 11$ ) groups. The ordinate gives the data as the percent of the basal diameters (table 4), where values less than 100% indicate constriction.

#### A1 RESPONSE TO SEROTONIN

Serotonin gave a comparable constriction of large (A1) arterioles in both the PB-CON and ETO-CON groups (fig. 4). Basal diameters were similar among the groups (table 4). Blocking prostaglandin synthesis did not affect the response of the A1 arterioles to serotonin in either the mefenamic acid groups (PB-MEF and ETO-MEF, fig. 5) or in the ibuprofen groups (PB-IBU and ETO-IBU, fig. 6). There were no statistically different responses to serotonin-induced A1 constriction among any of the groups.

#### RESPONSE TO TOPICAL ETOMIDATE

In pentobarbital-anesthetized animals with an A3 dilator capacity of  $118 \pm 21\%$ , etomidate was applied topically by adding it to the cremaster bath (PB-ETO). The topical application of etomidate did not affect either small (fig. 7, top) or large (fig. 7, bottom) arteriolar diameters.

#### Discussion

The characteristics of an ideal intravenous anesthetic agent include stability in solution, rapid onset of action,

TABLE 4. Basal Diameters of the A1 Arterioles in Etomidate- and Pentobarbital-anesthetized Rats

Group	Diameter ( $\mu\text{m}$ )
ETO-CON	$108 \pm 4$
ETO-MEF	$94 \pm 5$
ETO-IBU	$104 \pm 4$
PB-CON	$108 \pm 6$
PB-MEF	$84 \pm 4$
PB-IBU	$92 \pm 9$

ETO = etomidate; CON = control; MEF = mefenamic acid; IBU = ibuprofen; PB = pentobarbital.

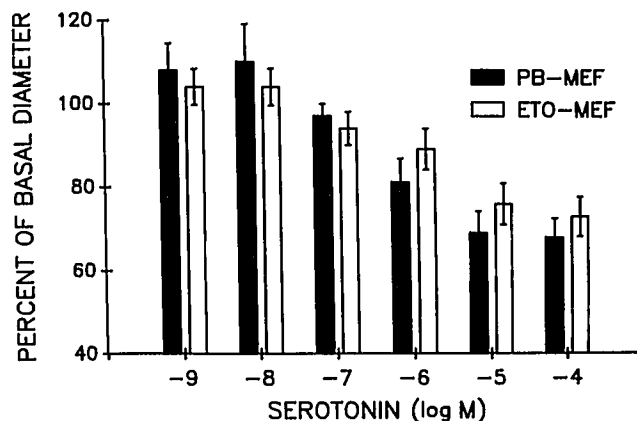


FIG. 5. The concentration-response curves to serotonin in large distributing arterioles (A1) in the pentobarbital-mefenamic acid ( $n = 6$ ) and etomidate-mefenamic acid ( $n = 6$ ) groups during cyclooxygenase inhibition with mefenamic acid. The ordinate gives the data as the percent of the basal diameters (table 4), where values less than 100% indicate constriction.

minimal effect on the cardiovascular and respiratory systems, short elimination half-life, and minimal side effects.<sup>23</sup> Using these criteria, the short-acting barbiturate sodium thiopental has long been considered the "gold standard" of intravenous agents used for human induction of anesthesia. A newer intravenous nonbarbiturate hypnotic agent, etomidate, has a rapid onset and short duration of action but less cardiovascular and respiratory depression than thiopental.<sup>24</sup> Well-known disadvantages of etomidate include pain on injection, myoclonus,<sup>9</sup> and suppression of cortisol production.<sup>25-28</sup> The effects of etomidate on major organ systems have been documented, but no published evidence was found regarding its actions

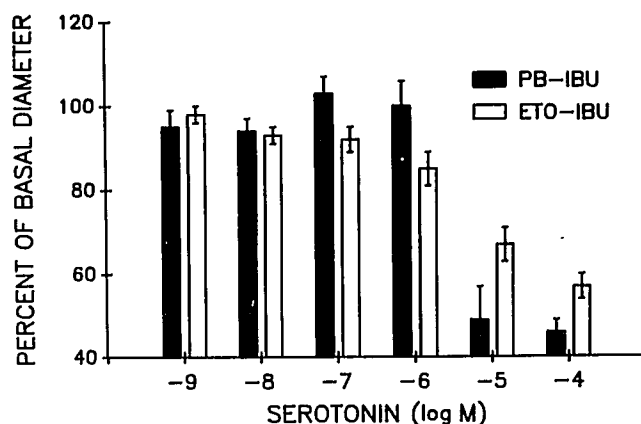


FIG. 6. The concentration-response curves to serotonin in large distributing arterioles (A1) in the pentobarbital-ibuprofen ( $n = 5$ ) and etomidate-ibuprofen ( $n = 6$ ) groups during cyclooxygenase inhibition with ibuprofen. The ordinate gives the data as the percent of the basal diameters (table 4), where values less than 100% indicate constriction.

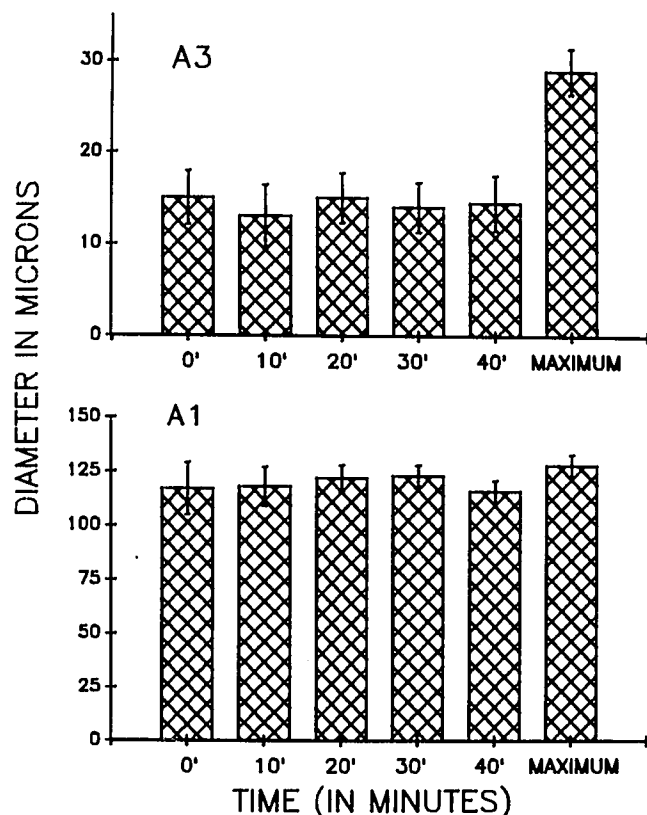


FIG. 7. The effect of the topical application of etomidate on the diameters of small (*top*) and large (*bottom*) arterioles ( $n = 5$ ) in pentobarbital-anesthetized rats (pentobarbital-etomidate group). The ordinate gives the data as the diameter in microns. Maximum refers to the maximally relaxed diameters achieved by the application of sodium nitroprusside.

in the microvasculature. Longnecker and Harris<sup>20</sup> previously demonstrated that microvascular diameters are altered by anesthetics, with the most profound alterations occurring in the smaller ( $<30\text{-}\mu\text{m}$ ) arterioles. These changes were tissue-specific and unique to each anesthetic agent. It was not known whether etomidate alters the sensitivity of microvessels to vasoactive agents. Previously, Miller and Wiegman have shown that the effective dose required for 50% of the maximal effect ( $ED_{50}$ , a measure of sensitivity) of the vasoconstrictor norepinephrine is altered by various anesthetics.<sup>29</sup> We chose to determine whether the sensitivity of the microvessels to serotonin was altered by etomidate. Serotonin has dual effects on the microcirculation. In urethane-chloralose-anesthetized rats, topical application of serotonin to the cremaster muscle causes large arterioles ( $>70\text{ }\mu\text{m}$ ) to constrict and smaller arterioles ( $<40\text{ }\mu\text{m}$ ) to dilate.<sup>30</sup> The same response has been demonstrated in pentobarbital-anesthetized rats, and antagonist studies indicate that the constriction and dilation are mediated by different serotonin receptors.<sup>14</sup> We selected serotonin for use in this study since both

vasodilator and vasoconstrictor sensitivity could be studied in the same preparation.

The depth of anesthesia appeared to be the same in the etomidate- and the pentobarbital-anesthetized rats. Arterial pressure and heart rate were steady (table 2); the animals did not respond to painful stimuli; and they had normal respiratory patterns and blood gas values (table 1). There were no differences between the two anesthetic groups in any of these physiologic parameters, giving indirect evidence that the activation of the sympathetic nervous system was similar in the two anesthetic groups. Although supplemental pentobarbital was given subcutaneously instead of intravenously to some animals (half of the PB-CON and all the PB-MEF animals), the arterial pressure, heart rate, and microvascular data were not affected by the route of supplemental anesthetic administration.

In the ETO-CON group, the small arterioles (A3) had very little dilator capacity; *i.e.*, the vessels possessed little basal tone. In contrast, the A3 arterioles in the PB-CON group had a very large dilator capacity ( $119 \pm 15\%$  greater than the basal diameter). The dilator capacity of the etomidate- or pentobarbital-anesthetized animals was not changed by the EDRF inhibitor nitro-L-arginine, indicating that EDRF was not involved in maintaining the basal arteriolar tone. In contrast, the dilator capacity of the A3 arterioles was increased in the ETO-MEF and ETO-IBU groups by inhibiting cyclooxygenase with either mefenamic acid or ibuprofen. In fact, the dilator capacity of the ETO-MEF and ETO-IBU groups was not different from that of the PB-CON, PB-MEF, or PB-IBU groups. Although the use of inhibitors alone is not enough to prove the involvement of a vasoactive substance in a response, these results do suggest that the basal dilation of the A3 arterioles in etomidate-anesthetized rats may be mediated *via* the release of dilator prostaglandins. Previous studies have shown that in the cremaster muscle, arachidonic acid dilates A3 arterioles by conversion to prostaglandin  $I_2$  and prostaglandin  $E_2$ .<sup>31</sup> Although mefenamic acid also inhibits lipoxygenase, ibuprofen acts primarily as a cyclooxygenase inhibitor<sup>32</sup>; therefore, prostaglandins are the most likely mediators of the etomidate-induced dilation observed in our study.

We also found that etomidate did not dilate cremasteric arterioles when applied topically (fig. 7), which suggests that etomidate does not act locally to cause A3 arteriolar dilation. This finding also suggests that etomidate causes the A3 dilation through an indirect mechanism, *i.e.*, *via* another endogenous agent or a breakdown product of etomidate.

An alternate explanation for the differences in dilator capacity between the ETO-CON and PB-CON groups is that pentobarbital caused arteriolar constriction of the small arterioles. This does not seem to be the case because

our pentobarbital-anesthetized animals had basal arteriolar diameters similar to those reported for decerebrate animals of the same weight range in a previous study.<sup>33</sup> Decerebration at the midcollicular level produces neurosurgical anesthesia. The decerebrated preparation most closely approximates the regional hemodynamic and skeletal muscle microvascular status of the awake rat.<sup>34,35</sup> In addition, the cremasteric A3 basal diameters reported in our current paper agree with those of rats anesthetized with urethane-chloralose,<sup>36</sup> halothane,<sup>33</sup> and Inactin.<sup>1</sup> Thus, the diameters of the pentobarbital-anesthetized animals are within the normal range of values for the decerebrate and anesthetized animal.

In etomidate-anesthetized rats, the constriction induced by serotonin of large (A1) arterioles was not different from that of the pentobarbital anesthetized group (fig. 4). Serotonin dilated A3 smaller arterioles (A3) in the PB-CON group but not in the ETO-CON group. In the ETO-CON group, serotonin had no dilator effect because these arterioles were already maximally dilated (table 3). When the dilator capacity was restored in the ETO-MEF and ETO-IBU groups, the dilation to serotonin was not different from that of the pentobarbital-anesthetized groups (figs. 2 and 3). Thus, the constrictor and dilator mechanisms in vascular smooth muscle appear to be unaltered by etomidate anesthesia. These data also show that the sensitivity to serotonin was similar in the etomidate- and pentobarbital-anesthetized animals.

We also were able to explore the role of prostaglandins in serotonin-induced vasoconstriction and vasodilation in the cremaster microcirculatory bed of pentobarbital-anesthetized rats. The A1 constriction to serotonin was not altered by the presence of mefenamic acid or ibuprofen with either anesthetic (figs. 5 and 6). In addition, serotonin-induced dilation in the PB-MEF and PB-IBU groups was not different from that of the PB-CON group. Thus, our data showed that prostaglandins were not involved in the serotonin-induced A1 constriction or A3 dilation in the cremaster muscle of pentobarbital- or etomidate-anesthetized rats. This finding agrees with an earlier study by Mayhan *et al.*<sup>37</sup> showing that serotonin-induced dilation of cerebral arterioles is not blocked by indomethacin in rats under pentobarbital anesthesia.

One finding of particular interest was the reaction of the small arterioles to systemic etomidate. Etomidate is known for its property of cardiovascular stability, yet it produced microvascular dilation in skeletal muscle that appeared to be a function of prostaglandins because it was blocked by mefenamate and ibuprofen. This was not

a local reaction to etomidate since etomidate did not cause the arterioles to dilate when it was applied topically to the cremaster muscle. Since cardiovascular parameters are relatively unchanged by etomidate,<sup>2-6,10,11</sup> it might be suggested that a compensatory vasoconstriction is occurring in another vascular bed. Etomidate is a potent direct vasoconstrictor of the cerebral vasculature.<sup>38</sup> In addition, Van Lambalgen and colleagues<sup>39</sup> reported that dogs anesthetized with etomidate-N<sub>2</sub>O had decreased blood flow to the gastrointestinal tract, liver, pancreas, skin, and skeletal muscle. However, the reduction in skeletal muscle blood flow did not occur until the 5th h of anesthesia. The duration of our experiments was only 2 h, a time at which Van Lambalgen *et al.*<sup>39</sup> reported no significant reduction in blood flow to any organ. In contrast, Prakash *et al.*<sup>2</sup> reported that etomidate-anesthetized pigs showed a decrease in renal and cerebral blood flow after 30 min of etomidate anesthesia. These studies indicate the most likely areas to be vasoconstricted during etomidate anesthesia of less than 2 h duration would be the renal and cerebral vascular beds.

In conclusion, etomidate alters arteriolar tone in skeletal muscle while preserving systemic hemodynamics in rats. If a similar effect was demonstrated to occur in humans, this property could be of potential clinical benefit, *i.e.*, in patients with peripheral vascular disease undergoing lower-extremity revascularization procedures or in patients requiring muscle flap coverage of soft tissue defects in which blood flow and stability of arterial pressure are critical to flap survival. Further studies would also be needed to assess the possibility and significance of a compensatory microvascular vasoconstriction occurring elsewhere in the body, concurrent to the dilation in skeletal muscle.

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