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Etomidate and Thiopental Inhibit the Release of Endothelium-derived Hyperpolarizing Factor in the Human Renal Artery

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Background: **Endothelium-derived hyperpolarizing factor is thought to be a cytochrome P450-derived arachidonic acid metabolite that hyperpolarizes vascular smooth muscle cells by opening Ca^{2+}-activated K^+ channels (K^+_{Ca} channels). In the rabbit carotid artery both volatile and intravenous anesthetics inhibit the acetylcholine-stimulated release of endothelium-derived hyperpolarizing factor. Because the release of this factor may help to maintain vascular tone in humans under conditions of a failing nitric oxide synthesis, *e.g.*, in atherosclerosis, the effects of two intravenous anesthetics, thiopental and etomidate, on the endothelium-derived hyperpolarizing factor-mediated relaxant response to acetylcholine were investigated in human isolated renal artery segments.**

Methods: **The segments were suspended in Krebs-Henseleit solution (37°C) containing the cyclooxygenase inhibitor diclofenac (1 μM) and preconstricted with norepinephrine (6 μM). Relaxations caused by acetylcholine (1 μM) were compared in the presence and absence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine (0.1 mM) in control segments and in segments exposed to etomidate or thiopental (0.03–0.3 mM). In addition, the effects of the two anesthetics on the relaxant response to the nitric oxide donors glyceryl trinitrate (3 μM) and sodium nitroprusside (0.1 μM) were examined.**

Results: **The relaxant response to acetylcholine, which was resistant to both nitric oxide synthase and cyclooxygenase blockade, was markedly reduced by the K^+_{Ca} channel antagonist tetrabutyl ammonium (3 mM) and the cytochrome P450 inhibitor clotrimazole (30 μM). Both etomidate and thiopental, at a concentration of 0.3 mM, selectively attenuated the relaxant response to acetylcholine in N^G-nitro-L-arginine-treated segments, but did not affect relaxations elicited by glyceryl trinitrate or sodium nitroprusside.**

Conclusions: **Etomidate and thiopental inhibit the endothelium-derived hyperpolarizing factor-mediated relaxant response to acetylcholine in the human renal artery, an effect that appears to be attributable to the cytochrome P450-inhibiting properties of these anesthetics. (Key words: Anesthetics, intravenous: etomidate; thiopental. Anesthetics, gases: nitric oxide. Arteries: renal. Heart, endothelium: endothelium-dependent relaxation; endothelium-derived hyperpolarizing factor. Enzymes: cytochrome P450. Neurotransmitters: acetylcholine.)**

IN addition to the release of nitric oxide and prostacyclin, agonists that increase the intracellular concentration of Ca^{2+} in endothelial cells, such as acetylcholine, dilate blood vessels by releasing a third endothelial autacoid that hyperpolarizes vascular smooth muscle cells and therefore has been termed endothelium-derived hyperpolarizing factor (EDHF). The release of this factor is associated with an increase in the K^+ conductance of the smooth muscle cells, an effect that is selectively blocked by antagonists of Ca^{2+}-activated K^+ channels (K^+_{Ca} channels).[1] Pharmacologic evidence is now accumulating that in certain vascular beds EDHF may be a cytochrome P450-derived arachidonic acid metabolite, presumably an epoxide.[2–7]

We have recently reported that both volatile[6] and intravenous anesthetics[7] inhibit the acetylcholine-stimulated release of EDHF, but not that of nitric oxide, in the rabbit carotid artery. Endothelium-derived hyperpolarizing factor release often is revealed only after blockade of nitric oxide synthesis, probably because nitric oxide exerts an intrinsic inhibitory effect on the synthesis of EDHF in the endothelium.[8] The failing nitric oxide synthesis mimicked by this experimental approach also occurs in humans, *e.g.*, in the carotid, coronary, and renal arteries, and is usually associated with prevalent cardiovascular diseases such as atherosclerosis. Under these circumstances, agonist-stimulated EDHF release, which appears to be largely intact,[1] may help to prevent the occurrence of vasospasm.[9]

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To verify the potential adverse effects of anesthetics on the release of EDHF in humans, we have investigated the effects of etomidate and thiopental on the EDHF-mediated relaxant response to acetylcholine in the human isolated renal artery. Although acetylcholine is unlikely to play a role as an endogenous vasodilator under physiologic conditions, the dilator response to this agonist is frequently assessed in patients with cardiovascular disorders as a parameter for the functional integrity of the endothelium.

Materials and Methods

Renal Artery Preparation

Branches of the main renal artery were isolated from the kidneys of 23 patients undergoing nephrectomy due to kidney carcinoma. The procedure was approved by the Human Investigation Committee of the University Clinic. The segments were cleaned of adipose and connective tissue, cut into rings 3–4 mm wide, and mounted between K30 force transducers (Hugo Sachs Elektronik, March, Germany) and a rigid support for measurement of isometric force. They were incubated in 10-ml organ chambers containing warmed (37°C) oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution, *p*H 7.4 (composition in mM: Na^+ 144.0, K^+ 5.9, Cl^- 126.9, Ca^{2+} 1.6, Mg^{2+} 1.2, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, HCO_3^- 25.0, and D-glucose 11.1) to which the cyclooxygenase inhibitor diclofenac was added at a concentration of 1 μM. Passive tension was adjusted during a 30-min equilibration period to 4 g, and the Krebs-Henseleit solution was exchanged at 10-min intervals. Stretching of the segments to this length was determined to result in an optimal isometric force development (80% of the maximum) in the presence of a fixed concentration (6 μM) of norepinephrine (9.8 ± 0.8 g tension, $n = 23$). Thereafter, the integrity of the endothelium was tested by applying 1 μM acetylcholine.

Experimental Protocol

After a washout period of 30 min, the rings were preconstricted again with norepinephrine, in the absence or presence of etomidate, thiopental, tetrabutylammonium, or clotrimazole at the concentrations indicated later. Acetylcholine was applied when a stable constriction was obtained. Two of the four rings did not receive any test compound, but were exposed to acetylcholine at the same time as the other two rings (time control). To study the effects of the anesthetics on the nitric oxide/prostacyclin-independent relaxant response to acetylcholine, in addition to diclofenac the segments were treated with N^G-nitro-L-arginine (100 μM) for 30 min followed by the same experimental protocol as described earlier. After another 30-min washout period, the segments were again constricted with norepinephrine, and the effects of the anesthetics on the endothelium-independent relaxation induced by glyceryl trinitrate or sodium nitroprusside were investigated in the same manner. Despite the repeated exposure to acetylcholine no tachyphylaxis was observed in any of the control segments throughout the study ($n = 23$).

Data Analysis

Unless indicated otherwise, all data in the figures and text are expressed as means ± SEM of n experiments (double determination) with ring segments from different arteries. Statistical analysis was performed with a two-sided Student's t test with a P value <0.05 considered statistically significant.

Materials

Diclofenac (Voltaren) was obtained from Ciba-Geigy (Wehr, Germany); N^G-nitro-L-arginine from Serva (Heidelberg, Germany); acetylcholine, clotrimazole, norepinephrine, and sodium nitroprusside from Sigma (Deisenhofen, Germany); thiopental (Trapanal) from Byk Gulden (Konstanz, Germany); etomidate (Hypnomidate) from Janssen (Neuss, Germany); and glyceryl trinitrate from Pohl-Boskamp (Hohenlockstedt, Germany).

Results

In some renal artery segments acetylcholine (0.03–10 μM) elicited a concentration-dependent relaxation, however, only application of the near maximal concentration of 1 μM produced a consistent relaxant response that was absent in endothelium-denuded rings (Fig. 1A). Treatment with N^G-nitro-L-arginine attenuated the acetylcholine-induced relaxation by 30–40%. This nitric oxide/prostacyclin-independent relaxant response was abolished in the presence of tetrabutylammonium (Fig. 1B) or Krebs-Henseleit solution containing 60 mM K^+ ($n = 4$). Clotrimazole also significantly attenuated the acetylcholine-induced relaxation in N^G-nitro-L-arginine-treated rings (Fig. 1C).

As in the rabbit carotid artery,[7] thiopental and etomidate (0.03–0.3 mM) did not affect the relaxant re-

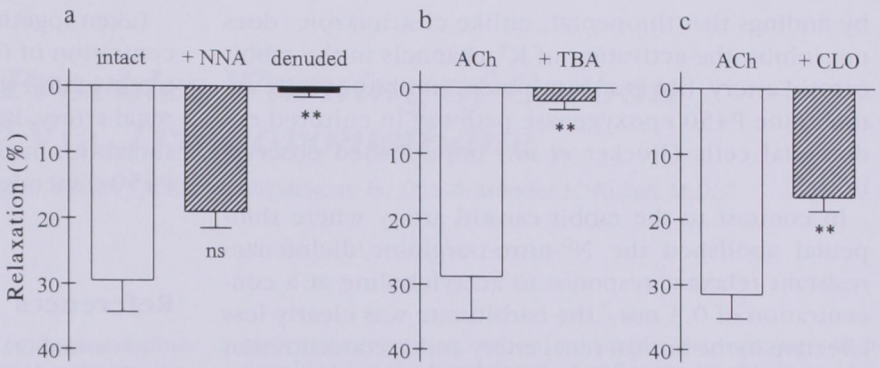

Fig. 1. (*A*) Relaxant response to 1 μM acetylcholine (ACh) in endothelium-intact segments, in the absence (intact) and presence of N^G-nitro-L-arginine (+NNA, 100 μM, $n = 12$), and in endothelium-denuded segments ($n = 10$). (*B*) and (*C*) Effects of tetrabutylammonium (+TBA, 3 mM, $n = 6$) and clotrimazole (+CLO, 30 μM, $n = 11$) on the N^G-nitro-L-arginine/diclofenac-resistant relaxant response to acetylcholine ($P < 0.01$ *vs.* control; ns, not significant).**

sponse to acetylcholine in control segments ($n = 4$), but exerted a significant inhibitory effect in N^G-nitro-L-arginine-treated rings at a concentration of 0.3 mM (Figs. 2A and 2B). The two anesthetics elicited a weak inhibitory effect (10–20% inhibition) already at a concentration of 0.1 mM, but this effect did not gain statistical significance (not shown). Thiopental (figs. 2C and 2D) and etomidate ($n = 5$, not shown) also had no significant effect on the endothelium-independent relaxant response to glyceryl trinitrate or sodium nitroprusside at all concentrations tested.

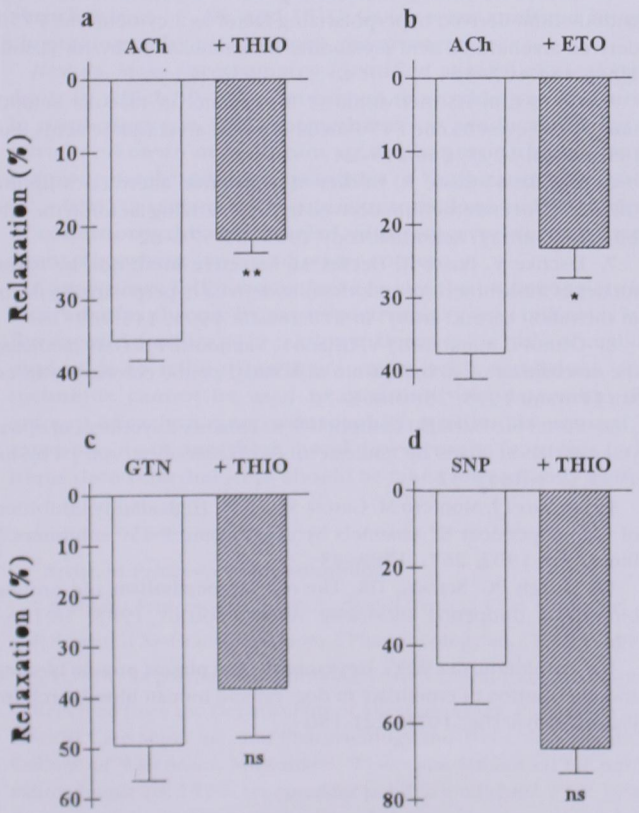

Fig. 2. (*A*) and (*B*) Effects of thiopental (+THIO, 0.3 mM, $n = 12$) and etomidate (+ETO, 0.3 mM, $n = 7$) on the N^G-nitro-L-arginine/diclofenac-resistant relaxant response to acetylcholine (ACh). (*C*) and (*D*) Effects of thiopental (+THIO, 0.3 mM) on the relaxant response to 3 μM glyceryl trinitrate (GTN, $n = 5$) and SNP sodium nitroprusside (0.1 μM, $n = 5$) in segments treated with N^G-nitro-L-arginine (*$P < 0.05$, **$P < 0.01$ *vs.* control; ns, not significant).

Discussion

The current findings demonstrate that in addition to nitric oxide an EDHF-like factor contributes to a large extent (60–70%) to the relaxant response to acetylcholine in the human renal artery. This N^G-nitro-L-arginine/diclofenac-insensitive relaxation was virtually abolished in the presence of tetrabutylammonium or after raising the extracellular K^+ concentration to 60 mM, suggesting that it was mediated by a hyperpolarization of the smooth muscle cells through activation of K^+_{Ca} channels. Although the finding that clotrimazole inhibited the acetylcholine-induced relaxation in N^G-nitro-L-arginine-treated segments points to the involvement of cytochrome P450 in the synthesis of EDHF in the human renal artery, other effects of clotrimazole, for example, a nonselective inhibition of K^+ channels,[10] cannot be ruled out.

However, at a concentration of 0.3 mM, thiopental and etomidate selectively inhibited the EDHF-mediated relaxant response to acetylcholine to a similar extent as clotrimazole. Both anesthetics are effective cytochrome P450 inhibitors,[7] suggesting that they exert this effect by attenuating the cytochrome P450-dependent synthesis of EDHF in the endothelium rather than by interfering with the activation by EDHF of K^+ channels in the smooth muscle. This notion is supported

by findings that thiopental, unlike clotrimazole, does not inhibit the activation of K^+ channels in the rabbit carotid artery, but is an equipotent inhibitor of the cytochrome P450 epoxygenase pathway in cultured endothelial cells (Hecker *et al.*, unpublished observation).

In contrast to the rabbit carotid artery where thiopental abolished the N^G-nitro-L-arginine/diclofenac-resistant relaxant response to acetylcholine at a concentration of 0.3 mM,[7] the barbiturate was clearly less effective in the human renal artery at this concentration (only 40% inhibition). This may indicate that (1) a noncytochrome P450 metabolite contributes to the acetylcholine-induced relaxation in the human renal artery, which is resistant to nitric oxide synthase and cyclooxygenase blockade, or (2) the endothelial cytochrome P450 epoxygenase pathway in this vascular bed has a different susceptibility to inhibition by thiopental than the corresponding enzyme(s) in the rabbit carotid artery. The latter hypothesis is supported by the finding that thiopental has virtually no inhibitory effect on the bradykinin-induced release of EDHF in the porcine coronary artery (Hecker *et al.*, unpublished observation).

When compared to the peak plasma concentration of both anesthetics during the induction of general anesthesia in humans (thiopental, 0.05–0.3 mM[11]; etomidate, 8.3–25.0 μM[12]), the concentration of thiopental required to significantly inhibit the EDHF-mediated relaxant response to acetylcholine appears to be clinically relevant whereas that of etomidate is too high by approximately one order of magnitude. Moreover, both thiopental (85%) and etomidate (75%) are extensively bound to plasma proteins, so that their free plasma concentration usually does not exceed 50 μM or 6.3 μM, respectively.[11,12] Conversely, it is likely that both anesthetics accumulate in or at the vessel wall, and in particular within the endothelium, to a concentration that could match or even exceed that found to be effective in our organ bath model of the human vasculature.

Taken together, these findings suggest that at a concentration of 0.3 mM, thiopental and etomidate selectively inhibit the release of EDHF in the isolated human renal artery. Based on previous results, this action presumably is mediated by interfering with the cytochrome P450-dependent synthesis of this autacoid.

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