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Activation of α_{2B}-Adrenoceptors Mediates the Cardiovascular Effects of Etomidate

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Background: **The intravenous anesthetic etomidate exhibits structural similarities to specific α_2-adrenoceptor agonists of the type such as dexmedetomidine. The current study was performed to elucidate the possible interaction of etomidate with α_2-adrenoceptors in mice lacking individual α_2-adrenoceptor subtypes (α_2-KO).**

Methods: **Sedative and cardiovascular responses to etomidate and the α_2-agonist, dexmedetomidine, were determined in mice deficient in α_2-receptor subtypes. Inhibition of binding of the α_2-receptor antagonist [^{3}H]RX821002 to recombinant α_2-receptors by etomidate was tested in human embryonic kidney (HEK293) cells *in vitro*.**

Results: ***In vivo*, loss and recovery of the righting reflex required similar times after intraperitoneal injection of etomidate in wild-type and in α_{2A}-receptor–deficient mice, indicating that the hypnotic effect of etomidate in mice does not require the α_{2A}-receptor subtype. Intravenous injection of etomidate resulted in a transient increase (duration 2.4 ± 0.2 min) in arterial blood pressure in wild-type mice (17 ± 3 mmHg). Etomidate did not affect blood pressure in α_{2B}-KO or α_{2AB}-KO mice. In membranes from HEK293 cells transfected with α_2-receptors, etomidate inhibited binding of the α_2-antagonist, [^{3}H]RX821002, with higher potency from α_{2B}- and α_{2C}-receptors than from α_{2A}-receptors (K_i α_{2A} 208 μM, α_{2B} 26 μM, α_{2C} 56 μM). In α_{2B}-receptor–expressing HEK293 cells, etomidate rapidly increased phosphorylation of the extracellular signal-related kinases ERK1/2.**

Conclusions: **These results indicate that etomidate acts as an agonist at α_2-adrenoceptors, which appears *in vivo* primarily as an α_{2B}-receptor–mediated increase in blood pressure. This effect of etomidate may contribute to the cardiovascular stability of patients after induction of anesthesia with etomidate.**

ETOMIDATE (R-(+)ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate) is a potent, short-acting hypnotic that was introduced as an anesthetic in 1972.[1] Major advantages described with the use of etomidate are its hemodynamic stability and the minimal respiratory depression.[2,3] This pharmacologic profile renders etomidate particularly suitable for induction of anesthesia in critically ill patients and patients with cardiovascular disease. The anesthetic effect is thought to be mediated primarily through an action on γ-aminobutyric acid receptors.[4–6] In addition, interactions of etomidate with second messenger systems such as the nitric oxide metabolism have been shown.[7] However, similar to other general anesthetics, the exact mechanism of its action remains to be shown.

The carboxylated imidazole etomidate exhibits structural similarities to specific α_2-adrenoceptor agonists that belong to the class of imidazole compounds, such as clonidine and dexmedetomidine.[8,9] Besides the chemical structure, etomidate and α_2-adrenoceptor agonists share some clinical similarities, such as inducing sedation/hypnosis with high cardiovascular stability and only minor respiratory depression.[2,10,11] In accordance with these findings, previous *in vitro* and *in vivo* studies indicate that the anesthetic action of etomidate might at least be partially mediated by α_2-adrenoceptors.[12]

Three subtypes of α_2-adrenoceptors, termed α_{2A}, α_{2B}, and α_{2C}, have been cloned from several species including mice and humans.[13–15] In both species, α_2-receptor subtypes are encoded by distinct genes which are localized on separate chromosomes. To further elucidate the role of α_2-adrenoceptors in the effects of etomidate, we studied the interaction of etomidate with α_2-adrenoceptor subtypes *in vivo* in mice carrying targeted deletions of α_2-receptor genes[16] and *in vitro* in human embryonic kidney (HEK293) cells expressing individual murine α_2-receptor subtypes.[17]

Materials and Methods

Generation and Genotyping of α_2-Adrenoceptor–deficient Mice

The generation of the mouse lines lacking single α_2-adrenoceptor subtypes has been described previously.[18,19] Mice lacking α_{2A}- and α_{2B}-receptors (α_{2AB}-KO) were generated by crossing of single gene knockout lines to first obtain double heterozygous mice, which were further intercrossed until homozygous α_{2AB}-deficient mice were born. Genotypes were confirmed by subtype-specific polymerase chain reactions performed with genomic DNA isolated from small tail biopsies as described in detail.[20] Mice were maintained in a specified pathogen-free facility. The University of Würzburg and the Government of Unterfranken (Würzburg, Germany) approved all animal procedures (protocol No. 621–2531.01–28/01).

Sedation/Hypnosis Induced by Etomidate

Mice were given an intraperitoneal injection of etomidate (5–50 mg/kg body weight), thiopental (50 mg/kg body weight), or dexmedetomidine (500 μg/kg or

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1 mg/kg), and the time after injection at which the righting reflex of mice was lost and the recovery time of this reflex were monitored. Because dexmedetomidine at these doses did not induce a loss of the righting reflex, its sedative effect was also assessed by placing mice on a rotating wheel (30 rpm) and determining the time for which mice stayed on the rod (Rotarod; Ugo Basile, Varese, Italy). For these series of experiments, male and female mice (3–4 months old, 5–10 mice per genotype) were used. The observer was blinded with respect to the genotype of the mice.

Blood Pressure Recording and Cardiac Catheterization

For hemodynamic measurements, two groups of male mice were used at the age of 3–5 months. In the first group, the cardiovascular effects of intravenous dexmedetomidine (5 μg/kg) were determined (eight mice per genotype). The second group was used to measure hemodynamic parameters after etomidate injection (6–10 mice per genotype). For aortic and left ventricular catheterization with a 1.4F pressure-volume catheter,[21] mice were anesthetized with tribromoethanol (13 μl of 2.5% solution per g of body weight) and placed on a 37°C table. The microtip catheter was inserted into the right carotid artery and the pressure tip was advanced into the aorta or into the left ventricle. For injection of drugs, a PE-10 polyethylene tubing was inserted into the left jugular vein. Etomidate or dexmedetomidine were injected in 0.9% saline into the jugular vein catheter. Hemodynamic data were digitized *via* a MacLab system (AD Instruments, Castle Hill, Australia) connected to an Apple G4 PowerPC computer (Apple Computer, Inc., Cupertino, CA).[21]

Cell Culture

Stable cell lines of human embryonic kidney (HEK293) cells with high expression levels of either the cloned murine α_{2A}-adrenoceptor (14.6 pmol receptor/mg protein) or α_{2C}-adrenoceptor (16.8 pmol receptor/mg protein) subtype were grown to near confluency in cell culture at 37°C.[17] In addition, because a stable cell line expressing the α_{2B}-adrenoceptor subtype was not available, native HEK293 cells were transiently transfected with the cloned mouse α_{2B}-adrenoceptor subtype by the calcium phosphate precipitation technique[22] and used 48 h after transfection. Transient transfection of α_{2B}-adrenoceptors resulted in expression levels that were similar to the stable cell lines.

Initially, HEK293 cells were washed with 5 ml of phosphate-buffered saline. The cells were then scraped from the culture dishes into 5 ml of ice-cold hypotonic buffer (5 mM Tris, 2 mM EDTA, pH 7.4) and washed again with 5 ml of ice-cold hypotonic buffer. Cells were homogenized with an Ultra-Turrax (Janke & Kunkel, Staufen, Germany) and cell nuclei and whole cells were removed by centrifugation at 1,700*g* for 10 min at 4°C. Membrane vesicles were prepared by centrifugation of the supernatant at 145,000*g* for 30 min at 4°C. The resulting final membrane pellet was resuspended in binding buffer (75 mM *N*-methyl-D-glucamine, 25 mM glycine, 40 mM HEPES, 5 mM EGTA, 10 mM MgCl, pH 7.4). Protein concentrations were determined using a colorimetric assay based on the method of Bradford[23] with bovine serum albumin as a reference standard.

Competition Binding Studies

Competition binding of etomidate to α_2-adrenoceptors was determined in a 200-μl reaction containing 10 nM to 1 mM etomidate, the α_2-adrenoceptor antagonist [^{3}H]RX821002 (3 nM), and binding buffer. Nonspecific binding was determined by addition of the α_2-receptor antagonist atipamezole (1 μM). The 200-μl reactions containing only the solvent propylene glycol without etomidate were used as controls. Membranes (10–20 μg protein) were incubated at room temperature for 1 h in the binding mixture assay. The binding reaction was stopped by filtration using a cell harvester and three washes of buffer. Membrane-bound [^{3}H]RX821002 was determined by scintillation counting.

Mitogen-activated Protein Kinase Phosphorylation

To determine the agonist activity of etomidate, untransfected or α_{2B}-receptor–transfected cells were stimulated for 20 min with 10 μM etomidate. Before stimulation, HEK cells were incubated overnight in serum-free Dulbecco's Modified Eagle Medium. Stimulated HEK cells were rapidly scraped off the plates and frozen in liquid nitrogen. Cell lysates were prepared and used for Western blotting as described previously.[20] Antibodies against the extracellular signal-related kinases ERK1/2 and phospho-ERK1/2 were purchased from Cell Signaling (Beverly, MA).

Pharmaceutical Compounds

The α_2-antagonist [^{3}H]RX821002 was used as a radioligand with a specific activity of 67 Ci/mmol (Amersham Pharmacia Biotech, Freiburg, Germany). Nonspecific binding was determined using the antagonist atipamezole (Orion Corp., Turku, Finland). Etomidate was either used as Hypnomidate® (2 mg etomidate/ml dissolved in 35% propylene glycol in water; Janssen-Cilag, Neuss, Germany) or as Etomidate®-Lipuro (2 mg etomidate/ml liposome suspension; Braun, Melsungen, Germany). Propylene glycol was used as the inactive control solution.

Statistics

The results of the competition binding were analyzed using a nonlinear regression program. A binding curve was fitted according to a one-site competition model (Prism; GraphPad, San Diego, CA). Data are presented as

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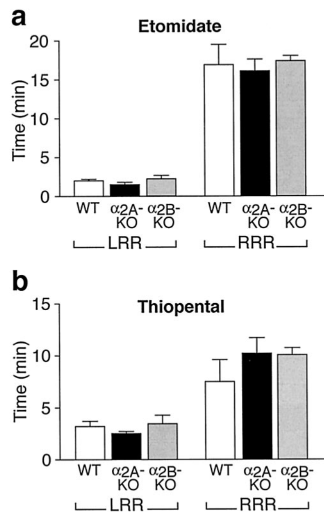

Fig. 1. Hypnotic effects of etomidate and thiopental in wild-type (WT), α_{2A}-receptor–deficient (α_{2A}-KO), and α_{2B}-receptor–deficient (α_{2B}-KO) mice. Etomidate (30 mg/kg) or thiopental (50 mg/kg) were intraperitoneally injected into male mice (n = 5 per genotype) and the time until loss of righting reflex (LRR) and recovery of the reflex (RRR) were monitored. Deletion of the α_{2A}- or α_{2B}-receptor genes did not influence the hypnotic effects of etomidate or thiopental.

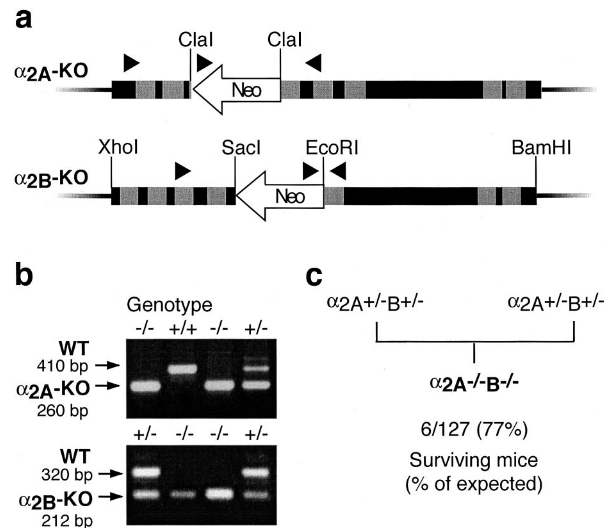

Fig. 2. Generation of mice lacking α_{2A}-adrenergic (α_{2A}-KO) and α_{2B}-adrenergic (α_{2B}-KO) receptors. (*a*) Schematic representation of the murine α_{2A}- and α_{2B}-KO alleles that were targeted for deletion by insertion of a neomycin cassette. *Gray areas* indicate location of the sequences encoding for transmembrane regions of the α_2-receptors. *Arrowheads* show location of polymerase chain reaction primers used for genotyping. (*b*) Representative polymerase chain reactions to detect wild-type (WT) and KO alleles for the α_{2A} (*upper panel*) and the α_{2B}-receptor (*lower panel*). (*c*) α_{2AB}-KO mice were derived from crosses of double heterozygous mice at the expected Mendelian frequency.

geometric mean with 95% confidence intervals. From the competition curves, K_i values were calculated from EC_{50} values using the Cheng Prusoff equation.[24] Hemodynamic data were analyzed using Student *t* test for unpaired samples. A *P* value of less than 0.05 was considered as statistically significant. Results are displayed as means ± SEM.

Results

Etomidate-mediated Hypnosis/Sedation

In vivo, the sedative effect of α_2-receptor activation is almost exclusively mediated by the α_{2A}-receptor subtype.[25] Thus, to test whether the sedative/hypnotic effect of etomidate is mediated *via* the α_{2A}-receptor, the times for the loss and for recovery of the righting reflex were determined in wild-type mice and in mice lacking α_{2A}- or α_{2B}-receptors (α_2-KO). For these experiments, intraperitoneal etomidate doses between 5 mg/kg and 50 mg/kg were tested in wild-type mice. Whereas 5 mg/kg etomidate did not result in loss of the righting reflex, 27% of the mice lost the reflex at 10 mg/kg etomidate and all mice transiently lost the righting reflex at 20-mg/kg and higher etomidate doses. Thus, we chose a dose of 30 mg/kg intraperitoneal etomidate to investigate sedative effects of etomidate in α_2-receptor-KO mice. After injection of etomidate, the righting reflex disappeared in wild-type and α_{2A}-deficient mice at similar times after intraperitoneal injection (fig. 1a). The duration of etomidate-induced sleep was not affected by the α_{2A}-deletion and the righting reflex recovered at similar times in wild-type and α_{2A}-KO mice. Similarly, hypnotic properties of intraperitoneal thiopental (50 mg/kg) did not differ between wild-type and α_{2A}-KO mice (fig. 1b). Deletion of the α_{2B}-receptor gene did not affect the sedative effects of etomidate or thiopental in mice (fig. 1). Intraperitoneal injection of the non–subtype-selective α_2-agonist dexmedetomidine up to 1 mg/kg did not result in a loss of the righting reflex. Thus, the sedative effect of dexmedetomidine was assessed on a rotating rod. Dexmedetomidine exerted a sedative effect in wild-type mice and in α_{2B}-KO mice, as evidenced by the observation that the mice were unable to stay on a rotating rod for longer than 10–20 s (wild-type, 9 ± 2 s; α_{2B}-KO, 14 ± 6 s; n = 8 mice per genotype, 30 min after 500 μg/kg intraperitoneal dexmedetomidine). In contrast, α_{2A}-KO mice did not show any sedative effects in response to dexmedetomidine (α_{2A}-KO stayed on the rod for more than 60 s, n = 10 mice).

Hemodynamic Effects of Etomidate in α_2-Receptor-deficient Mice

To test potential effects of etomidate on cardiovascular α_2-receptor subtypes, we first generated double-knockout mice deficient in α_{2A}- and α_{2B}-receptors (fig. 2 a and b). The α_{2AB}-deficient mice were generated by crossing single receptor knockouts to obtain double heterozygotes that were further crossed to yield mice with the $\alpha_{2A}-/-_{B}-/-$ genotype (fig. 2c). At weaning age, α_{2AB}-KO mice were detected at a frequency that was close to the expected Mendelian ratio, indicating that genetic deletion of these receptors did not interfere with embryonic devel-

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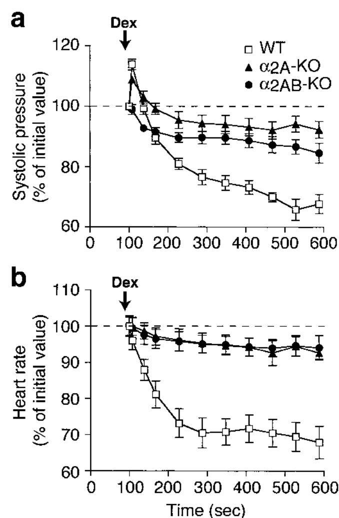

Fig. 3. Hemodynamic responses of α_{2A}-receptor–deficient (α_{2A}-KO), and α_{2AB}-receptor–deficient (α_{2AB}-KO) mice to dexmedetomidine (Dex). Systolic aortic pressure (*a*) and heart rate (*b*) were recorded in mice during tribromoethanol anesthesia. Intravenous dexmedetomidine (5 μg/kg) resulted in a biphasic pressure response that was accompanied by a rapid bradycardia (n = 8 mice per genotype). WT = wild type.

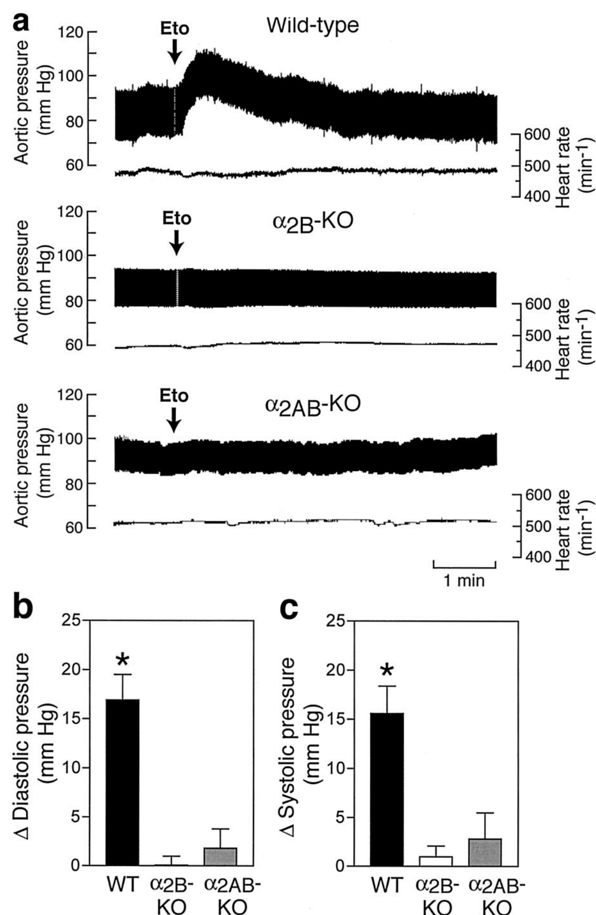

Fig. 4. Cardiovascular effects of etomidate (Eto) in α_{2A}-receptor–deficient (α_{2A}-KO), and α_{2AB}-receptor–deficient (α_{2AB}-KO) mice. (*a*) Representative aortic pressure traces recorded in anesthetized mice. Intravenous etomidate (2 mg/kg) caused a rapid and transient rise in blood pressure in wild-type mice that was not observed in α_{2B}- or α_{2AB}-deficient mice. (*b,c*) Significant increases in diastolic (*b*) or systolic (*c*) blood pressure were observed in wild-type (WT) animals but not in mice lacking α_{2B}- or α_{2AB}-receptors. *P < 0.05 etomidate *versus* baseline (n = 6–10 mice per genotype).

opment. After weaning, α_{2AB}-deficient mice developed normally and showed no obvious phenotypes.

First, we determined basal cardiovascular parameters in anesthetized α_{2AB}-KO mice. Mean arterial pressure did not differ between α_{2AB}-deficient animals (85 ± 4 mmHg, n = 9) and wild-type mice (89 ± 6 mmHg, n = 11). However, α_{2AB}-KO mice had a higher resting heart rate (493 ± 13 min^{-1}, n = 9) than wild-type control mice (446 ± 13 min^{-1}, n = 11). The cardiovascular response to the α_2-receptor agonist was tested in anesthetized mice of different genotypes. As reported previously, rapid intravenous injection of dexmedetomidine resulted in a transient hypertension in wild-type mice that was followed by a long-lasting hypotension (fig. 3a). Deletion of the α_{2A}-receptor disrupted the hypotensive response, whereas the initial hypertension was absent in α_{2B}-deficient animals. Interestingly, α_{2AB}-KO mice showed a small but significant decrease in systolic blood pressure (−13 ± 4 mmHg). This pressure effect was accompanied by a decrease in heart rate (−25 ± 8 min^{-1}, fig. 3b).

On intravenous injection of etomidate, wild-type mice showed a rapid and transient hypertensive response that was completely absent in mice lacking α_{2B}-receptors (fig. 4). During the hypertensive phase, heart rate decreased by 18 ± 6 min^{-1} in wild-type mice. No significant alterations in heart rate were observed in α_{2B}-KO or α_{2AB}-KO mice. After intravenous etomidate, mean arterial blood pressure remained above the preinjection value for 2.4 ± 0.2 min. The hypertensive effect of etomidate in wild-type mice was dose dependent: 1 mg/kg etomidate caused a small increase in blood pressure (systolic pressure, +6.7 ± 2.6 mmHg; diastolic pressure, +5.7 ± 2.2 mmHg; n = 6), whereas a dose of 2 mg/kg etomidate elicited significantly larger pressure responses (fig. 4; systolic pressure, +15.7 ± 2.7 mmHg; diastolic pressure, +17.0 ± 2.6 mmHg).

Two different preparations of etomidate, one in propylene glycol and the other in liposome suspension, showed identical effects on blood pressure in wild-type mice. Etomidate did not alter cardiac contractility as assessed by direct catheterization of the left ventricle. Maximal left ventricular contraction rate after intravenous injection of 2 mg/kg etomidate was identical (dp/dt$_{max}$ 101.6 ± 2.1% of control) to the value before etomidate.

Binding of Etomidate to Recombinant α_2-Adrenoceptors

To confirm the *in vivo* data, we next tested whether etomidate interacts with recombinant α_2-receptor subtypes expressed in HEK293 cells. For these experiments, cells expressing 14–18 fmol/mg of the three α_2-receptor subtypes were used. Etomidate inhibited binding of the

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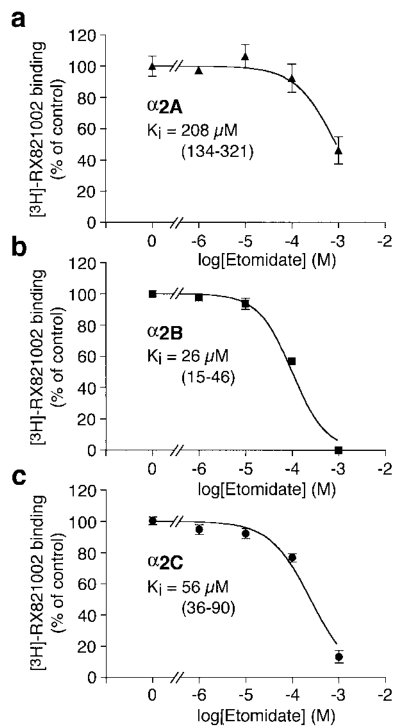

Fig. 5. Displacement of [^{3}H]RX821002 from α_2-receptor subtypes by etomidate. The α_{2A}-, α_{2B}-, or α_{2C}-receptors were expressed in HEK293 cells as described in Materials and Methods. Competition binding of etomidate with the radioligand [^{3}H]RX821002 was monitored in membranes prepared from α_2-receptor–expressing cells. Etomidate competed for [^{3}H]RX821002 binding with higher affinity at α_{2B}- and α_{2C}- than α_{2A}-receptors. Values in brackets are 95% confidence intervals obtained from six independent experiments performed in triplicate for each receptor subtype.

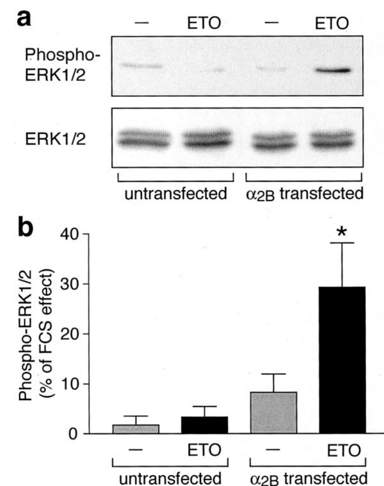

Fig. 6. Etomidate activates mitogen-activated protein kinase phosphorylation *via* α_{2B}-receptors. (*a*) ERK1/2 activation was detected in HEK293 cells expressing α_{2B}-receptors using phosphorylation-specific ERK1/2 antibodies (*upper panel*) or conventional antisera to detect total ERK1/2 levels (*lower panel*). Stimulation with 10 μM etomidate (ETO) for 20 min caused a significant increase in phospho-ERK1/2 levels in α_{2B}-receptor–transfected cells that was not observed in untransfected HEK293 cells (*b*). *Asterisk* indicates a significant difference ($P < 0.05$) with etomidate *versus* control; n = 6 from three independent experiments. ERK1/2 = extracellular signal-related kinases.

specifically bound α_2-receptor antagonist [^{3}H]RX821002 in a concentration- and subtype-dependent manner with greater potency for α_{2B}- and α_{2C}-receptors than for α_{2A}-adrenoceptors (fig. 5).

To test whether etomidate acted as an agonist or antagonist at α_{2B}-receptors, the effect of etomidate on α_2-receptor–mediated mitogen-activated protein kinase activation was assessed in HEK293 cells. Among other intracellular signaling pathways, α_2-receptors can effectively activate the mitogen-activated protein kinase cascade.[20] In untransfected or α_{2B}-receptor–expressing HEK293 cells, basal phosphorylation of the ERK1/2 kinases was hardly detectable (fig. 6). However, short-term exposure to etomidate increased ERK1/2 phosphorylation in HEK293 cells transfected with α_{2B}-receptors but not in untransfected cells (fig. 6).

Discussion

The present results indicate that etomidate interacts with α_2-adrenoceptors and displaces the α_2-adrenoceptor antagonist [^{3}H]RX821002 in HEK293 cells from all subtypes in a concentration-dependent manner. In addition, etomidate leads to a transient increase in blood pressure in wild-type mice, but not in α_{2B}-KO or α_{2AB}-KO mice, suggesting an interaction of etomidate with α_{2B}-adrenoceptors in the peripheral vasculature.

α_2-Adrenoceptors have been implicated in a variety of physiologic functions. Pharmacologic studies and the development of genetic mouse models elucidated the physiologic effects mediated by the different α_2-adrenoceptor subtypes.[16,18,26,27] The α_{2A}-adrenoceptor subtype has been reported to be the predominant subtype involved in the antinociceptive, sedative, hypotensive, hypothermic, and behavioral actions of α_2-adrenoceptor agonists.[25,26,28,29] Stimulation of α_{2B}-adrenoceptors in vascular smooth muscle leads to vasoconstriction, which causes the initial hypertension after administration of α_2-adrenoceptor agonists.[18] In addition, the α_{2B}-adrenoceptor subtype is involved in mediation of the antinociceptive action of nitrous oxide.[30,31] The α_{2C}-receptor has been shown to modulate dopaminergic neurotransmission, various behavioral responses, and to induce hypothermia.[32,33] In addition, this subtype contributes to spinal antinociception of the imidazoline moxonidine in mice.[34]

The exact molecular targets by which etomidate mediates its anesthetic effects remain under discussion. Clinically relevant plasma concentrations of etomidate to induce hypnosis vary considerably. During induction of anesthesia in humans, initial plasma concentrations of etomidate may be as high as 12–24 μM.[35] These concentrations are similar to the K_i value of etomidate for α_{2B}-receptors expressed in

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HEK293 cells in our study. Etomidate concentrations from 1.2–8.2 μM have been reported as the minimal hypnotic plasma levels for anesthesia in humans.[35] It has been shown that etomidate at these concentrations exhibits γ-aminobutyric acid–modulatory effects, and at higher concentrations exhibits γ-aminobutyric acid–mimetic effects at γ-aminobutyric acid receptor type A receptors.[4,5,36,37] Interestingly, mice with a point mutation in the $\beta3$ subunit of the γ-aminobutyric acid receptor type A receptor did not show any suppression of noxious-evoked movements in response to etomidate.[6] In this study, 5 mg/kg etomidate was injected intravenously in wild-type mice to elicit a loss of the reflex, which lasted for approximately 10 min.[6] In our study, we observed a significant transient hypertensive response to intravenous injection of 2 mg/kg etomidate. Thus, higher doses of etomidate may even cause a more pronounced hypertensive phase in mice. However, in the other study, the mice were already anesthetized with tribromoethanol for arterial catheterization and we did not use higher doses of etomidate to prevent toxicity. The low affinity of etomidate for the α_{2A}-adrenoceptor subtype determined in the current study challenges the assumption that the anesthetic effects of etomidate could be partially mediated by an action on cerebral α_2-adrenoceptors. Indeed, we found no difference in the sedative/hypnotic effect of etomidate between normal mice and animals lacking functional α_{2A}-adrenoceptors.

Our results show a higher affinity of etomidate for α_{2B}- and α_{2C}-adrenoceptor subtypes expressed in HEK293 cells. The role of the α_{2C} adrenoceptor subtype in anesthesia and cardiovascular control is currently still unknown. Previous investigations in mice have shown that disruption of the α_{2C}-adrenoceptor subtype does not lead to hemodynamic effects.[18] However, α_{2C}-receptors operate together with α_{2A}-receptors as presynaptic inhibitory regulators of sympathetic norepinephrine release.[21] To unequivocally distinguish the cardiovascular roles of α_{2C}- and α_{2B}-receptors *in vivo*, we generated mice deficient in α_{2A}- and α_{2B}-receptors. Interestingly, these mice showed a small but significant decrease of the heart rate in response to the α_2-agonist dexmedetomidine, which was accompanied by a decrease in aortic blood pressure. Administration of dexmedetomidine failed to produce the initial hypertension, which can be attributed to the α_{2B}-adrenoceptor subtype[18] and the central hypotensive effect related to the α_{2A}-adrenoceptor subtype.[26] These findings are consistent with the observation that activation of presynaptic sympathetic α_{2C}-receptors can decrease norepinephrine release.[21]

Etomidate elicited a transient increase in aortic blood pressure in anesthetized wild-type mice that was not observed in α_{2B}-KO or in α_{2AB}-KO mice. The hypertensive response was accompanied by a mild bradycardia. In humans, the most distinctive property of etomidate as compared with other anesthetics is its minimal effect on cardiovascular parameters.[2,3] However, several studies in patients have shown that etomidate may cause transient increases in blood pressure and/or systemic vascular resistance.[38,39] Thus, during induction of anesthesia etomidate may interact with vascular α_{2B}-adrenoceptors to elicit a transient hypertensive effect or to counteract the hypotensive effects of other anesthetics and drugs used in anesthesia. In addition to a direct effect on vascular α_{2B}-receptors, etomidate may elicit the transient hypertension by an indirect mechanism. It is tempting to speculate that increased levels of catecholamines (caused by etomidate) may contribute to the transient hypertension. However, wild-type or α_2-deficient mice did not show any typical signs of increased norepinephrine secretion; *i.e.*, tachycardia (fig. 4). Further studies on isolated microvessels are required to determine whether the etomidate-mediated hypertension is indeed mediated by a direct vasoconstrictory mechanism.

Interaction of etomidate with the α_{2A}-receptor subtype is unlikely to play an important role in mediating its anesthetic action because of the low affinity of etomidate for this receptor subtype. This result is also consistent with the clinical profile of etomidate in humans. Activation of α_{2A}-receptors elicits analgesia and inhibits sympathetic outflow. Etomidate does not have significant analgesic effects in humans and it does not block the sympathetic activation that is usually observed during intubation.[40]

In conclusion, etomidate can stimulate α_{2B}-adrenoceptors in mice, which results in a transient hypertensive response after rapid intravenous injection. Although this study was performed with transgenic mouse models and recombinant murine α_2-adrenoceptors, it is tempting to speculate about the implications for the mechanism of action of etomidate in humans. The cardiovascular stability of etomidate during induction of anesthesia even in patients with cardiac disease and during bolus application might be related to an interaction of etomidate with peripheral α_{2B}-adrenoceptors. The α_{2B}-mediated vasoconstriction may oppose hypotensive effects of other coadministered agents during induction of anesthesia, and thus be responsible for the known beneficial hemodynamic profile of etomidate.

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