Neonatal Lethality in Mice Deficient in XCE, a Novel Member of the Endothelin-converting Enzyme and Neutral Endopeptidase Family*

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XCE is the latest member of the zinc metallopeptidase family, which includes the endothelin-converting enzymes ECE-1 and ECE-2, NEP (neutral endopeptidase or enkephalinase), Kell blood group antigen (KELL), and PEX (for a review, see Ref. 1). The corresponding cDNA was cloned recently from human caudate nucleus and spinal cord cDNA libraries (2). It encodes a type II transmembrane protein of 775 amino acids with a short cytosolic tail of 59 residues and a large luminal domain that contains the characteristic zinc-binding motif HEXXH. XCE shares the highest homology with ECE-1 (53% homology and 42% identity over the last 500 amino acids), but in contrast to ECE-1 it is only present as a single transcript. Expression in various cell types revealed a 95-kDa glycosylated protein consistent with the presence of three putative sites for Asn-linked glycosylation in its cDNA. XCE mRNA is preferentially expressed in the central nervous system (CNS).1 In human Northern blot experiments, highest expression was found in putamen, medulla, subthalamic nucleus, and spinal cord. In the rat and human CNS, a very specific pattern of neuronal labeling in presumptive cholinergic interneurons of basal ganglia, basal forebrain neurons, as well as brainstem and spinal cord motor neurons was detected by in situ hybridization histochemistry. In contrast, other brain regions including cerebellum and cerebral cortex were not labeled. Among the peripheral tissues, strong hybridization signals were observed in rat uterine subepithelial cells and around renal blood vessels. For all previously known members of the NEP/ECE family, either a function or a clinical importance has been described. NEP, which is identical to the common acute lymphoblastic leukemia antigen or CALLA (3–5), metabolizes many substrates including enkephalins, tachykinins, atrial natriuretic factor, and bradykinin (6). ECE-1 (7–12) is mainly responsible for the conversion of the inactive precursors, big endothelins, to the biologically active endothelins that play an important role in the maintenance of vascular tone but also in cell proliferation, hormone production, and various developmental processes. ECE-2 was also shown to convert big endothelins to endothelins but only under acidic conditions (pH 5.5) (13). Mutations in the PEX gene are responsible for the inherited renal disorder X-linked hypophosphatemic rickets (14), and parathyroid hormone-derived peptides have recently been identified as a first substrate for PEX (15). Although Kell (16, 17) is still an “orphan enzyme,” it is clear that Kell blood group antigen incompatibility can be associated with severe problems including hemolytic disease of the newborn.

The sequence similarity between XCE and members of the NEP/ECE family, in particular the presence of the zinc binding motif HEXXH and the conservation of several amino acids that are crucial for the enzymatic activity in NEP and ECE-1, suggests that XCE functions as a membrane-bound metallopeptidase. The enzyme substrate, as yet unidentified, might be found among the numerous neuropeptide transmitters that colocalize with acetylcholine in the XCE-positive neurons of the CNS. In the present study, we disrupted the XCE gene by homologous recombination and created XCE-deficient mice to gain insight into the importance and physiological role of XCE. We demonstrate that the deletion of XCE results in neonatal lethality and have investigated potential anatomical, skeletal, and histological abnormalities.

MATERIALS AND METHODS

Isolation of Genomic Clones and Construction of a XCE Targeting Vector—Genomic clones of XCE were obtained by screening a λ FIXH bryonic day; RT, reverse transcription; PCR, polymerase chain reaction; TK, thymidine kinase; HSV, herpes simplex virus; bp, base pair(s); kb, kilobase pair(s).
To generate the targeting vector, the polylinker of the Bluescript vector (Stratagene) was first modified by insertion of two complementary oligodeoxynucleotides encoding XhoI-HindIII-BamHI-Sall-NorI restriction sites into the unique XhoI and NorI sites of pBSK resulting in the vector pBSK poly1. The herpes simplex virus (HSV) gene for thymidine kinase (TK) driven by the HSV-TK promoter was cloned into the XhoI and HindIII sites of this pBSK poly1 giving plasmid pBSK poly1-TK. The 5′-flanking arm consisting of a 1956-bp XhoI genomic fragment (recloned as XhoI-HindIII fragment) was then inserted together with a neomycin resistance cassette (excised as XhoI-BamHI fragment from pMC1 neo; Stratagene) downstream of the TK into the HindIII and BamHI sites of pBSK poly1-TK in a tripartite ligation (pBSK poly1-TK-XCE'-neo). In a last step, an approximately 7-kb HindIII genomic XCE fragment (recloned as Sall-NorI fragment) was inserted downstream of the neomycin resistance cassette into the Sall and NorI sites of pBSK poly1-TK-XCE'-neo to generate the 3′-flanking arm. In the resulting targeting vector (see Fig. 1A), the 1076 bp of XCE sequence between the two flanking arms including the coding sequence for the zinc binding motif HELTH is replaced by the 1087 bp neomycin resistance cassette. The construct was linearized at its unique NorI site prior to transfection.

**Generation of Mice with Targeted Allele—**An E14 ES cell line was grown on a feeder layer of irradiated neomycin-resistant mouse embryonic fibroblasts in standard ES medium (Dulbecco’s modified Eagle’s medium containing 15% fetal calf serum, 0.1 mM 2-mercaptoethanol, 2 mM glutamine, 1× nonessential amino acids, and 1000 units/ml leukemia inhibitory factor (Life Technologies, Inc.).) Approximately 20×10⁶ ES cells were electroporated with 25 μg of the linearized targeting vector and selected for double resistance to G418 and ganciclovir (Life-neo). In a last step, an approximately 7-kb HindIII genomic XCE fragment (recloned as Sall-NorI fragment) was inserted downstream of the neomycin resistance cassette into the Sall and NorI sites of pBSK poly1-TK-XCE'-neo to generate the 3′-flanking arm. In the resulting targeting vector (see Fig. 1A), the 1076 bp of XCE sequence between the two flanking arms including the coding sequence for the zinc binding motif HELTH is replaced by the 1087 bp neomycin resistance cassette. The construct was linearized at its unique NorI site prior to transfection.

**Genotyping of Tail Biopsies—**Genotyping of postnatal day 7–14 mice, newborn pups, and embryos was performed by Southern blot analysis and/or polymerase chain reaction (PCR) (Qiagen, solution Q system). Genomic DNA was isolated from tail biopsies using proteinase K (18). Oligonucleotide primers used for PCR detection were ATGGCGTCACT-GGTGCCTTCTCC (P1), GGTTGTCCTGCAGGTTGG-3′ and 5′-GGTCGAGGTCATGCGITGG-3′ and 5′-GGGAC CTGCAAAAATGCTGAGGGTTG-3′ and 5′-TGCCAG-GCAAATGAAAGAAGCTAGG-3′, respectively. These primers were deduced from mouse sequence located 5′ (5th exon in Fig. 1A) and 3′ (11th exon in Fig. 1A) of the disrupted region (8th to 10th exon in Fig. 1A) and correspond to bp 1854–1880 and bp 2353–2392 of the human cDNA, respectively. For the semiquantitative RT-PCR analysis of surfactant-associated proteins, cDNA was amplified using the following primers: mouse SP-A (406-bp fragment), 5′-GATCGAAACATCGATCC-TGCAAAATCAATG-3′ and 5′-AAATCT CCATAGCGCTGCAAGC-3′, mouse SP-B (342-bp fragment), 5′-CTGGTCGCTGCTGTGAGTTGCTGG-3′ and 5′-GGTTCG GAGTTGCTGCAAGC-3′, mouse SP-C (452-bp fragment), 5′-TGTCCTGCGTTTGTGACCCAT-3′; mouse SP-D (403-bp fragment), 5′-GGTTG GCTTCTCTCCTCAATCGAAGCCTG-3′ and 5′-TCAGA AACTCACAATAAGGCGGTGTCCT-3′. Mouse β-actin (605-bp fragment) was analyzed as an internal control for normalization. Appropriate dilutions were determined for each cDNA preparation and amplified fragment (SP-A, SP-B, SP-C, SP-D, and β-actin) to ensure that the resulting PCR products were derived only from the exponential phase of the amplification. The reaction was cycled 26 times (30 s at 94°C, 30 s at 55°C, and 30 s at 72°C). Aliquots of the PCR reaction were subjected to

**Fig. 1** Disruption of the XCE gene. A, schematic representation of a segment of the wild-type mouse XCE gene, the targeting construct, and the targeted XCE allele. Solid black boxes represent the last 13 exons (coding sequence); the exon marked (*) encodes the zinc-binding motif of XCE “HELTH,” the neomycin resistance gene (neo) and the HSV-TK gene (TK) are shown. The neo cassette is flanked by 2 kb of 5′ and 7 kb of 3′ XCE genomic sequence. The Southern blot probe (hatched box) and PCR primers (arrows; P1–P3) used are shown. Restriction enzyme: B, BamHI. B, Southern blot analysis of DNA from wild-type and XCE mutant ES cells. Genomic DNA was digested with BamHI and hybridized as described above. C, RT-PCR analysis of brain RNA from XCE+/+ (864 bp), XCE+/− (864 and 410 bp), and XCE−/− (410 bp) E18.5 embryos. A litter of 9 animals is shown. D, RT-PCR analysis of brain RNA from XCE−/−, XCE+/−, and XCE−/− E18.5 mice. XCE-specific primers were deduced from sequence of the 5th and 11th exon shown in A.

**TC**CTCCACACAGGG-3′; and mouse SP-D (403-bp fragment), 5′-GGTTG GCTTCTCTCCTCAATCGAAGCCTG-3′ and 5′-TCAGA AACTCACAATAAGGCGGTGTCCT-3′. Mouse β-actin (605-bp fragment) was analyzed as an internal control for normalization. Appropriate dilutions were determined for each cDNA preparation and amplified fragment (SP-A, SP-B, SP-C, SP-D, and β-actin) to ensure that the resulting PCR products were derived only from the exponential phase of the amplification. The reaction was cycled 26 times (30 s at 94°C, 30 s at 55°C, and 30 s at 72°C). Aliquots of the PCR reaction were subjected to

**TCT**CCACACAGGG-3′; and mouse SP-D (403-bp fragment), 5′-GGTTG GCTTCTCTCCTCAATCGAAGCCTG-3′ and 5′-TCAGA AACTCACAATAAGGCGGTGTCCT-3′. Mouse β-actin (605-bp fragment) was analyzed as an internal control for normalization. Appropriate dilutions were determined for each cDNA preparation and amplified fragment (SP-A, SP-B, SP-C, SP-D, and β-actin) to ensure that the resulting PCR products were derived only from the exponential phase of the amplification. The reaction was cycled 26 times (30 s at 94°C, 30 s at 55°C, and 30 s at 72°C). Aliquots of the PCR reaction were subjected to
electrophoresis and the products visualized by ethidium bromide staining. The gel image was digitized with a video camera, and the intensity of the bands was quantitated with the ImageQuant software (Molecular Dynamics).

Evaluation of Mice—C-sectioned embryos on day 18.5 of gestation and newborn pups were first analyzed for external alterations. For visceral examinations, fetuses and pups from various litters were preserved and subsequently studied for soft tissue anomalies according to Barrow and Taylor (19). For the skeletal analysis, fetal E18.5 skeletons from several litters were processed with Alizarin Red S stain according to Dawson (20). For the determination of heart beat frequencies, C-sectioned embryos on day 12 of gestation were placed in prewarmed Williams’ Medium E (Life Technologies) containing 10% rat serum. The heart beats were counted three times for 1 min each.

Histotechnique—All animals and organs were fixed in 10% neutral buffered formalin. The following organs were embedded in paraffin wax, sectioned at 2–3 μm, and stained with hematoxylin-eosin for routine histological examination: Adrenal glands, aorta, brain, eyes, heart, mesenteric lymph nodes, kidneys, small and large intestine (duodenum, jejunum, ileum, cecum, colon, rectum) liver, lungs, esophagus, pancreas, salivary glands, skin, spleen, stomach, thymus, thyroid/parathyroid, urinary bladder, and trachea. Serial sections were performed of the brainstem and stained with hematoxylin-eosin or cresyl fast violet to demonstrate Nissl substance.

RESULTS

Targeted Disruption of the XCE Gene—We used homologous recombination in ES cells to generate mice lacking functional XCE. Genomic clones of the mouse XCE gene were isolated from a 129/sv mouse genomic library, mapped, and partially sequenced. The luminal domain of XCE contains the HEXXXH zinc-binding motif (HELTH), which is likely to be essential for its enzymatic activity. In a number of metallopeptidases including ECE-1 and NEP, mutation of either histidine or the glutamic acid of this motif resulted in a totally inactive enzyme (see Ref. 1 and references therein). To disrupt XCE in vivo, the exon encoding the zinc-binding motif and the next two downstream exons were replaced with a neomycin resistance cassette (Fig. 1A). A thymidine kinase gene was included at the 5’ end of the targeting vector as a negative selection marker against random integration. The targeting vector was electroporated into ES cells (derived from the 129/sv mouse strain), and clones resistant to G418 and gancyclovir were screened for homologous recombination events by Southern blot analysis. Genomic DNA was digested with BamHI and probed with a XCE gene fragment external to the targeting vector. The wild-type XCE allele gave an approximately 9-kb band, while the targeted allele resulted in a 3.3-kb fragment (Fig. 1B). The correct targeting event was detected in 25 out of 100 ES cell clones that were screened. Recombinant ES cells were injected into C57BL/6 mouse blastocysts, and resulting male chimeras

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** Selected fetal views demonstrating the absence of cardiovascular and craniofacial defects in XCE-deficient mice. A and B, gross photographs of the great vessels from E18.5 wild-type (A) and XCE−/− (B) embryos. A, aortic arch; da, ductus arteriosus; lc, left carotid; ls, left subclavia; rc, right carotid; rs, right subclavia; Pt, pulmonary trunk; t, trachea. C and D, alizarin-stained skeleton preparations of fetal heads from E18.5 wild-type (C) and XCE−/− (D) mice. M, mandible; b, basiaphenoïd; h, hyoid; o, odontoid; t, thyroid cartilage. The presence or absence of eye pigmentation is incidental in the two animals and not related to the gene disruption. The blue color in D is the remnant of an incomplete Alcian blue staining.

| Table I |

| Genotypes of the progeny from XCE (+/+ −/− intercrosses) |
| Genomic DNA was extracted from the tails of E18.5 embryos or neonates (1–2 weeks dpp) and then subjected to PCR analysis to determine the genotype. | No. of pups | +/+ | +/− | −/− | Knock-out % |
| Age | 18.5 dpc | 174 | 43 | 78 | 53 | 30 |
| 1–2 weeks dpp | 310 | 92 | 218 | 0 | 0 |

* XCE −/− (knock-out) mice calculated as the percentage of all genotyped mice in the same age group.

![Downloaded from http://www.jbc.org/](http://www.jbc.org/)
were bred to C57BL/6 females. Germline transmission of the disrupted allele resulted in heterozygous mice that developed normally, were fertile, and showed no obvious abnormalities.

Neonatal Lethality and Respiratory Defects in XCE \(-/-\) Homozygous Mice—To determine the effect of the targeted XCE allele in the homozygous state, heterozygous mice were intercrossed and the offspring were genotyped by Southern and PCR analysis. No viable homozygotes (XCE \(-/-\)) were found when 310 offspring were analyzed at 1–2 weeks of age (Table I). The ratio of wild-type to heterozygous progeny was approximately 1:2 (Table I). These findings indicate that XCE is an essential gene, disruption of which results in perinatal death.

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To confirm complete inactivation of the XCE gene, XCE expression was examined by RT-PCR. As expected, RT-PCR revealed no expression of the XCE gene in homozygous E18.5 XCE \(-/-\) embryos (Fig. 1D).

At birth all XCE \(-/-\) pups displayed the same striking characteristics. The newborn homozygotes could open their mouths but remained cyanotic and died of anoxia within 10–30 min. It is important to note that none of the XCE \(-/-\) homozygotes survived beyond this very early neonatal stage. The breathing problems were not due to an occlusion of the trachea, as demonstrated by probing of several embryos. Examination of their lungs, however, indicated that the vast majority were never ventilated.

Taken together, these results demonstrate that XCE \(-/-\) homozygous mice die shortly after birth from impaired breathing and suggest that XCE may be important in the control of respiration.

Anatomical, Skeletal, and Histological Characterization of XCE \(-/-\) Homozygous Mice—Given the normal gross appearance of E18.5 XCE \(-/-\) embryos and XCE \(-/-\) newborn, we next performed a thorough visceral examination of 57 E18.5 embryos (18 \(-/-\)) derived from 6 intercrosses of heterozygotes as well as of 20 newborn pups (11 \(-/-\)). Although we observed some anomalies that occurred in wild-type \((+/+)\), heterozygous \((+/-)\), and homozygous \((-/-)\) animals at about the same frequency, the study did not reveal any anatomical malformations specific for the XCE knock-out. As an example, a comparison of the great vessels from XCE-deficient and wild-type mice is shown in Fig. 2 (A and B).

To study the skeleton in the XCE knock-out animals, embryos from 7 litters obtained by C-section on day 18.5 (21 \(-/-\)) were analyzed and stained with Alizarin Red S. The skeletal and cartilage components of XCE \(-/-\) embryos did not show any abnormal characteristics that are related to the gene targeting (Table II). The examination revealed some retardations and variations that were, however, present in wild-type, heterozygous, and homozygous fetuses at about the same incidence, indicating that they are typical for the strain of mouse. The only remarkable skeletal malformations, which were detected, affect the sternum and also occurred with comparable frequency in wild-type, heterozygous, and homozygous embryos. It is worth noting that our skeletal analysis did also not detect any craniofacial abnormalities, such as the fusion of the thyroid cartilage, hyoid bone, and basi-epiphysis bone, which could contribute to the respiratory failure in XCE \(-/-\) homozygotes (Fig. 2, C and D).

To further characterize the XCE \(-/-\) animals, a histopathological evaluation of all major organs was performed. The examination of lungs obtained from several litters confirmed that the vast majority of lungs from XCE \(-/-\) mice remained atelectatic after birth (Fig. 3). Congenital atelectasis was found.

**Table II**

Summary of skeletal anomalies

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>No. of alive/dead</th>
<th>No. of male/female</th>
<th>No. of Fetuses</th>
<th>No. of Fetuses with retardations</th>
<th>No. of Fetuses with variations</th>
<th>No. of Fetuses with abnormalities</th>
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<td>(+/-)</td>
<td>(+/-)</td>
<td>(-/-)</td>
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<td></td>
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<tr>
<td>Fetuses with retardations</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Incompletely ossified bones</td>
<td>11</td>
<td>22</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Thoracic rib 13 rudimentary</td>
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<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Lumbar vertebra missing</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td>Sternal elements misshapen,</td>
<td>6</td>
<td>12</td>
<td>12</td>
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<td>fused, or misaligned</td>
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* Not all fetuses could be sexed.
in 15/20 homozygous animals analyzed. From the five non-atelectatic lungs, three were only minimally aerated. In contrast, the lungs of XCE\textsuperscript{1/1} mice were all properly inflated. Besides the atelectasis, the lung structures of the XCE\textsuperscript{2/2} animals did not show any obvious anomalies when compared with their wild-type littermates.

As mentioned above, XCE mRNA is preferentially expressed in the CNS where medulla, spinal chord, putamen, and subthalamic nucleus show highest levels of expression. This tissue distribution, together with the early respiratory failure of XCE\textsuperscript{2/2} animals, prompted us to study the CNS and especially the brain stem region with the medulla oblongata, known to be of importance in the regulation of vegetative functions, such as respiration, in more detail. To this end, serial sections of the brainstem were prepared and stained with hematoxylin-eosin and cresyl fast violet (Nissl). The histopathological examination did not reveal any obvious differences between XCE\textsuperscript{2/2} and wild-type control animals. In particular, no changes in morphology and distribution of neuronal cells were detected in the medulla oblongata of XCE\textsuperscript{2/2} mice (Fig. 4). Differences between mutant and wild-type animals were also not found in the other regions of the CNS including spinal chord and putamen.

In addition, the histological analysis did not detect apparent abnormalities in any other organs, such as heart, kidney, liver, eyes, intestine, and trachea.

**Analysis of the Heartbeat Frequency and the Expression of Pulmonary Surfactant Proteins in XCE\textsuperscript{−/−} Mice**—To extent the analysis of the XCE-deficient mice, we first compared the heart beat frequency of wild-type and XCE mutant embryos. No significant differences in the mean heart rate were detected between XCE\textsuperscript{+/+} (87 ± 14 bpm), XCE\textsuperscript{+/−} (83 ± 12 bpm), and XCE\textsuperscript{−/−} (81 ± 9 bpm) mice, indicating that the presence of XCE is not important for a normal heart beat frequency.

Postnatal respiratory failure can be due to a deficiency of pulmonary surfactant proteins (21, 22). To test whether this is the case in XCE knock-out animals, the expression of the surfactant-associated proteins SP-A, SP-B, SP-C, and SP-D was examined in lungs of wild-type and XCE\textsuperscript{−/−} E18.5 mice. Semiquantitative RT-PCR analysis revealed very similar mRNA levels of each of the four surfactant-associated proteins in XCE\textsuperscript{1/1} and XCE\textsuperscript{2/2} animals (Fig. 5). When the results of several experiments were quantitated, the expression in XCE-deficient mice was found to be 111 ± 11% of the wild-type level for SP-A, 92 ± 8% for SP-B, 97 ± 10% for SP-C, and 102 ± 7% for SP-D. Thus, the loss of XCE function does not cause a deficiency of pulmonary surfactant proteins.

**DISCUSSION**

In the present study, we disrupted the XCE gene in mouse ES cells by gene targeting and established mice bearing the mutant XCE allele. The resulting phenotype is characterized by neonatal lethality of 100% of the XCE\textsuperscript{−/−} homozygotes, demonstrating for the first time that XCE has a critical, non-redundant function.

The cause of death in the XCE-deficient animals appears to be anoxia due to respiratory failure. While observing female mice giving birth, we found that homozygous mutant neonates were born alive but died shortly thereafter. In contrast to their wild-type and heterozygous littermates, which turned pink...

**FIG. 4.** Histopathological sections of the brainstem from XCE\textsuperscript{−/−} and XCE\textsuperscript{+/+} mice. Histological sections of the brainstem, medulla oblongata, stained with hematoxylin/eosin. A and C, wild-type; B and D, XCE\textsuperscript{−/−} mouse. C and D, higher magnifications of A and B, including the Solitary nucleus (Sol). No obvious differences were apparent between the wild-type control animal and the XCE\textsuperscript{−/−} mouse.
within minutes, all XCE \(-/-\) newborns remained cyanotic. An examination of their lungs revealed that the vast majority were never ventilated. Other than atelectasis, no detectable pathological changes in the lung structures of XCE \(-/-\) mice were observed in histological sections, demonstrating that the respiratory distress is not intrinsic to this organ. The absence of obvious craniofacial abnormalities affecting the upper airway or mouth, the lack of thoracic malformations, as well as the absence of a tracheal occlusion or a fusion between craniofacial bones and the trachea indicate that the respiratory failure after birth is most likely not caused by mechanical obstruction. Another possible reason for impaired breathing can be the reduced production of surfactant proteins, a family of polypeptides in pulmonary alveoli implicated in lung function. Similar to the phenotype of XCE \(-/-\) mice, disruption of the surfactant protein B gene resulted in lethal perinatal respiratory distress associated with atelectasis (21, 22). However, no significant differences in the expression of the surfactant-associated proteins SP-A, SP-B, SP-C, and SP-D were found between XCE \(+/+\) and XCE \(-/-\) mice, indicating that a deficiency of pulmonary surfactant proteins is not the cause for the respiratory failure in XCE \(-/-\) animals. The same was true for the Clara cell 10-kDa protein (CC10), another pulmonary differentiation marker.\(^2\) The respiratory distress appears also not to be the result of cardiac problems since no anomalies of the heart or great vessels were found. Furthermore, the heart beat frequency was not significantly affected in XCE \(-/-\) mice. Thus, a dysfunction in the central respiratory control becomes a likely possibility for the observed lethality of the XCE \(-/-\) newborns. Spontaneous automatic respiration is controlled by the respiratory center in the medulla and transmitted via the spinal chord to motor neurons that control the respiratory muscles. In the rat, for example, phasic respiratory neurons have been located in the ventrolateral part of the medulla oblongata (23). This is particularly intriguing since the medulla and the spinal chord were among the tissues with the highest expression levels for the XCE mRNA, which is preferentially expressed in the CNS (24). Although we did not detect obvious abnormalities in the formation of the medulla oblongata or other structures of the CNS in XCE \(-/-\) mice by our histopathological examination, the possibility that subtle defects within these structures underlie the loss of vital functions will have to be investigated. For example, these may involve synaptic connections or transmission. The latter possibility is especially attractive since the specific neuronal expression pattern of XCE together with the fact that it belongs to a metallopeptidase family suggests that it may be involved in the maturation of one or several neuropeptide transmitters (2). An alternative explanation for the lethality of the XCE \(-/-\) animals, that we consider, however, less likely, are abnormalities in the respiratory muscles themselves, in particular the muscles of the diaphragm and the intercostal muscles.

Targeted disruption has previously been reported for other members of the NEP/ECE zinc metallopeptidase family. No developmental abnormalities have been detected in ECE-2-deficient mice (24). Mice lacking NEP also appeared developmentally normal except for some minor differences in lymphoid development. However, a strongly enhanced susceptibility to endotoxin shock was observed (25). ECE-1 null mice, on the other hand, showed a lethal phenotype (26). Approximately 75% of the ECE-1 \(-/-\) embryos died in utero, and the ECE-1 \(-/-\) term embryos all died within 30 min of birth from impaired breathing. The ECE-1 \(-/-\) mice exhibited severe craniofacial and cardiac abnormalities comparable to the defects seen in ET-1 \(-/-\) (27) and endothelin receptor A (ET\(_A\)) \(-/-\) (28) homozygotes. In addition, enteric nerves and epidermal melanocytes are absent in the ECE-1-deficient embryos, recapitulating the developmental changes found in endothelin receptor B (ET\(_B\)) \(-/-\) (29) and ET-3 \(-/-\) (30) mice. Although XCE \(-/-\) homozygous animals share the respiratory failure with the ECE-1 \(-/-\), ET-1 \(-/-\), and ET\(_A\) \(-/-\) mice, we did not detect any of the neural crest-related malformations seen in these animals. For example, none of the craniofacial abnormalities that could narrow the upper airway were found. The observed differences in the phenotypes between XCE \(-/-\) mice and knock-out animals of the major proteins of the endothelin system support our recent evidence that XCE does not appear to play a role in this system (2).

In conclusion, the generation of XCE null mice has given insight into the physiological importance of XCE. The disruption of XCE in mice has proven to be incompatible with life after birth. Although further investigations are clearly necessary to precisely the exact mechanism of the lethality, our results suggest that XCE may play an essential role in the regulation of the respiratory system.

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