Uncoupling protein-1: involvement in a novel pathway for β-adrenergic, cAMP-mediated intestinal relaxation

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Shabalina, Irina, Claes Wiklund, Tore Bengtsson, Anders Jacobsson, Barbara Cannon, and Jan Nedergaard. Uncoupling protein-1: involvement in a novel pathway for β-adrenergic, cAMP-mediated intestinal relaxation. Am J Physiol Gastrointest Liver Physiol 283: G1107–G1116, 2002. The pathway for adrenergic relaxation of smooth muscle is not fully understood. As mitochondrial uncoupling protein-1 (UCP1) expression has been reported in cells within the longitudinal smooth muscle layer of organs exhibiting peristalsis, we examined whether the absence of UCP1 affects adrenergic responsiveness. Intestinal (ileal) segments were obtained from UCP1-ablated mice and from wild-type mice (C57Bl/6, 129/SvPas, and outbred NMRI). In UCP1-containing mice, isoprenaline totally inhibited contractions induced by carbachol; isoprenaline; forskolin

SMOOTH MUSCLE TONE IS ACHIEVED by a balance between the actions of contraction- and relaxation-inducing agents. Intracellular signaling pathways for the corresponding processes have been proposed (for reviews see Refs. 38, 43, 53, 65, 73). The pathway from the muscarinic acetylcholine receptors to enhanced contraction clearly involves an increase in cytosolic Ca2+ levels. The corresponding pathway leading from stimulation of β-adrenergic receptors and increases in cAMP to relaxation of phasic smooth muscle (36, 24, 73) is not fully understood. It involves a reduction in Ca2+ levels but also a reduction in the effectiveness of Ca2+ in invoking contraction; the mechanism behind this reduced effectiveness has not been clarified. A novel possibility for explaining the reduced Ca2+ effectiveness has recently arisen unexpectedly based on observations originating from studies in an apparently unrelated field, that of nonshivering thermogenesis. The phenomenon of classic adaptive nonshivering thermogenesis is presently understood molecularly as being the outcome of the activity of the archetypal mitochondrial uncoupling protein-1 (UCP1; thermogenin) (reviewed in Refs. 16, 35, 51, 60) originally identified in brown adipose tissue (20, 40) and demonstrated to be the only protein able to mediate nonshivering thermogenesis (17). UCP1 functions as a regulated transporter for proton equivalents over the mitochondrial membrane, leading to energy being released as heat instead of being captured in ATP. Until recently, UCP1 has been considered to be expressed only in brown adipose tissue (3, 25), to the extent that its occurrence in a given adipose cell has become the established criterion distinguishing brown and white adipose tissue (4). Conversely, no indication of UCP1 expression in other tissues has existed that could not be understood as being caused by contamination of extracts with brown adipocytes or being explainable based on the existence of the recently identified closely related proteins UCP2 or UCP3 (2). However, in a recent investigation, Nibbelink et al. (52) have reported that UCP1 is expressed very specifically in cells that seem to be included among the longitudinal smooth muscle layer of a series of organs, all characterized by exhibiting peristaltic motion: stomach, esophagus, small intestine, ureter origin, ductus deferens, and uterus. In the cells that apparently contain UCP1, the level of UCP1 protein was of a magnitude similar to that in brown adipocytes (52). UCP1 was not found in arterial walls; and although arterial walls contain smooth muscle cells, they do not demonstrate peristaltic movements.

Functionally, the UCP1 could be present in these organs for the same reason that it is in brown adipose

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tissue, i.e., for thermogenesis. However, in classic experiments, Depocas (8, 9) showed that norepinephrine-induced thermogenesis in cold-acclimated rats was unaffected by functional evisceration, demonstrating that at least at the systemic level, no contribution was observable from any thermogenic process in the abdomen. It is therefore an interesting possibility that, in these organs, UCP1 has a functional role that is not thermogenic. In brown adipose tissue, the activation of UCP1 (thermogenesis) is induced by β-adrenergic receptors and increased cAMP (75, 76). In different organs exhibiting peristaltic motion, β-adrenergic receptors also have a role: to induce relaxation/inhibition of contraction observed under these conditions (see DISCUSSION), we have here utilized UCP1-ablated mice to examine whether a UCP1-dependent component exists in β-adrenergically induced relaxation.

MATERIALS AND METHODS

Animals

The UCP1-ablated [UCP1(−/−)] mice were progeny of those described (10) in which the gene encoding UCP1 had been inactivated by homologous recombination with a deletion in which exon 2 and parts of exon 3 had been replaced by a neomycin resistance gene; in the brown adipose tissue of these mice, no UCP1 can be detected with polyclonal antibodies (10; and unpublished observations). The mice were bred in the institute and were phenotypically [e.g., hair color as well as body wt and growth rate on standard diet and at normal (24°C) animal house temperature] indistinguishable from mice of the C57Bl/6 strain, which were the donors of the blastocysts. Genetically, the UCP1(−/−) mice are infiltrated with 129/SvJ (from the embryonic stem cells) and 129/SvPas (to which the chimeras were bred). Thus for the present experiments, three types of UCP1-containing [UCP1(+/−)] mice were used: the C57Bl/6 strain (B&K Universal, Stockholm, Sweden), a 129/SvPas strain (Charles River, Cleon, France), and genetically outbred NMRI mice (B&K Universal). All animals were fed ad libitum (rat and mouse standard diet No. 1; BeeKay Feeds, B&K Universal), had free access to water, and were kept on a 12:12-h light-dark cycle. Mice of 8- to 10-wk age and of both sexes were acclimated (1 per cage) to 24°C for at least 2 wk before the start of the experiments.

Tissue Collection

Routinely on each experimental day, one wild-type and one UCP1(−/−) mouse (weighing 20–22 g) were anesthetized for 1–2 min by a mixture of 79% CO₂-21% O₂ and killed by cervical dislocation. Ileum (~10 cm) was removed 2cm above the ileocelecal junction and immediately placed in preoxygenated physiological salt solution composed of (in mM) 149 Na⁺, 2.9 K⁺, 1.8 Ca²⁺, 0.5 Mg²⁺, 144 Cl⁻, 23.8 HCO₃⁻, 0.4 H₂PO₄⁻, and 5.5 glucose and aerated with 5% CO₂ in O₂. Adjacent mesentery was gently cut away from the ileum segments. Ileal segments of ~1.0-cm length were cut, and suture needles were used to pass silicone-treated silk thread (5-0 size) through a single wall on each end of the ileal segment.

Organ Bath Studies

Routinely, eight ileal segments (4 from each type of mice) were mounted vertically in 25-ml water-jacketed organ baths made of glass (Section of Engineering, Mayo Clinic) filled with the salt solution detailed above, maintained at 37°C, and bubbled continuously with 5% CO₂ in O₂. During the experiments, the solution in the baths was changed at 20- to 30-min intervals by superfusion of aerated solution preheated to 37°C. The segment preparations were given an initial isometric load of 2.5 mN (250 mg weight) and were allowed to equilibrate for 2–3 h before the experiments were started. We observed that the time required to obtain stable responses to electrical field stimulation (EFS) (see EFS) appeared to be longer in segments from UCP1(−/−) mice, but as experiments were routinely performed in parallel with segments from wild-type and UCP1(−/−) mice, the preconditioning time was the same. The motor activity was recorded isometrically by Grass force-displacement transducers (FT03) and tracings were made on a polygraph (model 79, Grass Instruments) (72).

EFS

Isolated ileal segment preparations were submitted to EFS by use of a direct current amplifier (Section of Engineering, Mayo Clinic) triggered by a stimulator (model S44, Grass Medical Instruments) via two parallel platinum electrodes (10 × 50 mm) 8 mm apart. This type of stimulation leads to contraction; this contraction may be due to a direct electrical effect on the muscles or due to stimulation of the nerves (that may be both excitatory and inhibitory for contraction). In methodological preexperiments, pulse duration response, and voltage response curves were, therefore, produced to determine optimal stimulation variables and to identify the nerve-mediated stimulation (that is sensitive to Na⁺ channel block). For the pulse duration response study, EFS was applied at 18 V, 3 Hz in 5-s trains every 2 min, and the pulse duration was increased gradually from 0.01 to 5.0 ms. This was repeated in the presence of 0.3 μM of the fast neuronal sodium channel blocker tetrodotoxin. At 0.2-ms pulse duration, the responses were maximum, with negligible responses remaining in the presence of tetrodotoxin. Because no response was observed when voltage-sensitive Na⁺ channels were blocked, these responses were regarded as nerve mediated, and this stimulation duration was used throughout the study. For the voltage-response study, the EFS was applied at various voltages (5, 10, 15, 30 V) at 3 Hz in 5-s trains every 2 min. Pulse duration was increased gradually from 0.1 to 3.0 ms at each voltage. Responses to EFS with 0.2 ms and 18 V were regarded to be (supra)maximal and were used in this study. Thus, for the experiments reported here, EFS of 0.2-ms pulse duration, 3-Hz frequency, and 15-V amplitude (as measured in the tissue baths, 18 V at the output) were applied in 5-s trains every 2 min.

Effects of Relaxing Agents on EFS-Evoked Contraction

Ileal segments were exposed to EFS as described above for 30–60 min before agonists were added. The mean of the amplitudes of the stable responses to EFS (the last 4 before additions) for each segment was considered as the control.
value and set to 100%. Investigated agents (as indicated in the relevant figure legends) were added cumulatively to the tissue baths during the pause between contractions. The mean of the amplitudes of the two contractions after the addition of each drug concentration was measured and expressed in percentage of the control response. On the basis of these normalized segment responses, the mean values from all segments (3–4) from each mouse were calculated.

Effects of Relaxing Agents on Carbachol-Induced Contraction

Ileal segments were contracted submaximally with 1 μM of the acetylcholinesterase-resistant muscarinic receptor agonist carbachol. The contraction reached a stable level within 8 min and maintained a stable plateau for >30 min when not further affected (not shown). To investigate the relaxant action, specified agonists (as indicated in the figure legends) were added cumulatively during this plateau contraction to the organ baths with 4 min allowed for stabilization of the relaxation response to each concentration. At the end of each cumulative dose-response curve, the ileal segments were maximally relaxed with 15 μM of the nonspecific smooth muscle relaxant papaverine. Total carbachol-induced contraction was defined as the difference between the stable carbachol value and the value after papaverine and was set to 100% for each segment, and all responses were expressed as a percentage of this carbachol response. On the basis of these normalized segment responses, the mean values from all segments (3–4) from each mouse were calculated. After full relaxant response had been obtained, tissue was rinsed at 10-min intervals for ≤30 min, and a new series of responses was recorded in the same manner.

Data Analysis

Mean dose-response data obtained from each mouse as described above (mean of 3–4 segments) were analyzed for simple Michaelis-Menten kinetics with the curve-fit option of the KaleidaGraph application using the equation \( V(x) = 100% - V_{\text{max}} \cdot [x/(IC_{50} + x)] \) where \( x \) is the concentration of the substance investigated. The maximum volume (\( V_{\text{max}} \)) and IC\(_{50}\) values thus generated for each mouse were used for statistical analysis with Student’s unpaired t-test.

Chemicals

Isoproterenol hydrochloride (isoprenaline), papaverine hydrochloride, tetrodotoxin, carbamylcholine chloride (carbachol), and forskolin were purchased from Sigma (St. Louis, MO). CL-316,243 was a gift from E. Danforth. Isoproterenol was dissolved in 0.1 mM ascorbic acid. All other drugs were dissolved in distilled water, except forskolin, which was dissolved in DMSO. Drugs were added to the baths in volumes <1% of the total bath volume. The final concentration of DMSO itself (0.5%) showed no significant biological effects.

RESULTS

A UCP1-Dependent Component in Isoprenaline-Induced Intestinal Relaxation

EFS. In wild-type mice, EFS induced stable and reproducible contractions in segments of small intestine (Fig. 1A); according to our methodological preexperiments (see MATERIALS AND METHODS), these contractions represented the outcome of nervous stimulation.

**Fig. 1.** Effect of the \( \beta \)-adrenoceptor agonist isoprenaline on electrical field stimulation (EFS)-induced contractile responses of ileal segments from mitochondrial uncoupling protein-1 (UCP1; UCP1-containing [UCP1(+/+)]; A) and UCP1-ablated (−/−) (B) mice. Ileal segments were isolated in parallel from UCP1(+/+) and UCP1(−/−) mice, and their contractile activity was monitored as described in MATERIALS AND METHODS. Filled circles indicate the application of EFS. Isoprenaline was added cumulatively to the indicated concentrations (log M) to the organ bath. At the start of the “recovery,” the organ bath was rinsed with fresh physiological solution.
of the segments. The general β-adrenergic agonist isoprorenaline dose dependently and reversibly inhibited these contractile responses to EFS; at high isoprorenaline concentrations, the contractile responses were virtually abolished (Fig. 1A). Data for seven mice are compiled in Fig. 2A. As seen, isoprorenaline was consistently able to fully inhibit the electrically induced contraction in wild-type mice: at 1 μM isoprorenaline, only 2 ± 1% remained (mean value from 7 mice), and based on Michaelis-Menten analysis of data points, the remaining contraction at infinite isoprorenaline concentration was −2 ± 2%, with an IC₅₀ for isoprorenaline of 23 ± 3 nM (mean ± SE from 7 animals, each individually analyzed as illustrated for the mean values in Fig. 2A).

As illustrated in Fig. 1B, EFS could induce phasic contractions also in ileal segments from UCP1(+/−) mice; there were no observable differences in contractile amplitude or basal muscle tone between the segments from UCP1(+/+) and UCP1(−/−) mice (6.9 ± 0.9 mN per 1-cm segment in wild-type segments and 6.3 ± 0.7 mN per segment from UCP1(−/−) mice; means from 7 animals in each group). As seen, although isoprorenaline was able to diminish the EFS-induced contractions dose dependently and reversibly also in the segments from UCP1(−/−) mice, it did not totally eliminate these contractions even at the highest concentrations shown; even a 10-fold higher concentration (10 μM) (in independent experiments) was not able to fully eliminate the contractions (not shown). Data from seven UCP1(−/−) mice examined in this way are compiled in Fig. 2A. As seen, at 1 μM isoprorenaline there was still a significant contraction (15 ± 4%). Moreover, analysis of the dose-response curves for Michaelis-Menten kinetics indicated a clear residual, isoprorenaline-insensitive component in the intestine from UCP1(−/−) mice: even at infinite isoprorenaline concentration, 10 ± 4% (*P < 0.02 vs. wild type; Student’s unpaired *t*-test) of the contraction remained (cf. also the results in Figs. 3–5 for this residual contraction). Furthermore, the intestinal segments from UCP1(−/−) mice were more than threefold less sensitive to isoprorenaline, demonstrating an IC₅₀ of 81 ± 18 nM (vs. the 23 ± 3 nM in the wild type; *P < 0.01).

It was of importance to confirm that UCP1(+/+) strains other than the C57Bl/6 behaved qualitatively and quantitatively similarly under the experimental conditions utilized. In Fig. 2B, we have, therefore, compiled data from other UCP1(+/+) mice strains and show them together with the curve fits from Fig. 2A. Data from the 129/SvPas strain (part of the genetic background of the UCP1(−/−) mouse), as well as from outbred NMRI mice, are shown. As seen, these other UCP1(+/+) strains behaved indistinguishably from the UCP1(+/+) C57Bl/6 mice used as wild type in Figs. 1 and 2A, both in demonstrating a high isoprorenaline sensitivity and especially in reaching full isoprorenaline-induced inhibition of EFS-induced contractions. There was thus no indication that the inability of isoprorenaline to fully inhibit contraction in the intestines from the UCP1(−/−) mice, and the lower isoprorenaline sensitivity observed in these intestines, was strain-
Addition of increasing concentrations of isoprenaline led, again as expected (19), to full relaxation of the carbachol-induced contraction, to the extent that addition of the general relaxant papaverine did not elicit any further relaxation. As compiled in Fig. 3C, full relaxation (−4 ± 3%) was always reached at infinite isoprenaline concentrations, and isoprenaline had an IC$_{50}$ of 27 ± 9 nM (n = 5), i.e., a value identical to its IC$_{50}$ value for inhibition of EFS-stimulated contractions.

As exemplified in Fig. 3B, carbachol could also induce a stable contraction in ileal segments from UCP1-ablated mice, and there was no observable difference between the carbachol-induced contractile force in intestine from UCP1(+/+) and UCP1(−/−) mice (5.5 ± 0.5 mN per segment vs. 5.2 ± 0.3 mN; n = 6). However, isoprenaline was unable to fully relax intestine from UCP1(−/−) mice, whereas papaverine still induced full relaxation. From the compilation in Fig. 3C, it is seen that the residual relaxation was 7 ± 1% (P < 0.03 vs. wild type) even at infinite isoprenaline concentration, with an isoprenaline affinity (IC$_{50}$) of 37 ± 9 nM. Thus a qualitative difference existed between intestine from UCP1(+/+) and UCP1(−/−) mice, irrespective of whether the contraction was induced by EFS (probably through release of endogenous acetylcholine from the nerves) or by added carbachol.

**β$_3$-Adrenergic Stimulation**

Besides being prominent in brown and white adipose tissue (15), β$_3$-adrenoceptors are also expressed in the intestine (11, 23, 66). Although β$_1$- and β$_2$-adrenoceptors are also expressed in the intestine (26, 54, 61), β-adrenergically induced relaxation in the intestine is mainly mediated by the β$_3$-adrenoceptors (23, 37). Similarly, the β$_3$-receptors are also the β-receptors mainly responsible for norepinephrine-induced thermogenesis (75, 76) and for norepinephrine-induced UCP1 gene expression (59) in brown adipocytes. We therefore also examined the influence of UCP1-ablation on β$_3$-adrenoceptor-mediated relaxation.

The β$_3$-specific agonist CL-316,243 was not fully as efficient as the general β-agonist isoprenaline in inhibiting EFS-induced intestinal contraction; a residual response of 22 ± 1% remained in wild-type mice (Fig. 4A) even at infinite isoprenaline concentrations (mean from 5 mice). The affinity (IC$_{50}$) for CL-316,243 was 19 ± 4 nM (Fig. 4A). When CL-316,243 was used to inhibit EFS-induced contraction in intestine from UCP1(−/−) mice, the residual contraction was significantly greater (41 ± 6%; P < 0.04) than in wild-type mice; the IC$_{50}$ was 21 ± 6 nM (Fig. 4A). We examined whether there was any effect of UCP1 ablation on the expression of the β$_3$-receptors in the intestine, principally performed as in (1), but found no difference in expression (not shown).

Similar observations were made when relaxation of carbachol-precontracted ileal segments was induced with CL-316,243 (Fig. 4B). Here the residual contraction was 16 ± 3% in intestine from wild-type mice.

**Carbachol-induced contraction**. Stimulation of intestine from UCP1(+/+) mice with the stable acetylcholine analog carbachol, as expected (19), to persistent intestinal contraction, as exemplified in Fig. 3A.
and as high as 35/6% in intestine from UCP1(-/-) mice (P < 0.03; means ± SE from 5 mice); the IC50 was 33 ± 9 in the wild-type and 34 ± 8 in the UCP1(-/-) mice.

Thus the absence of UCP1 resulted in a similar degree of lack of relaxation potency, whether the relaxation was induced by a general β-receptor or by a β3-selective agonist.

cAMP-Induced Relaxation Involves a UCP1-Dependent Component

To investigate whether the UCP1-dependent relaxation necessitated direct β-receptor involvement, we examined whether such a UCP1-dependent component would also be visible if the relaxation was induced downstream from the receptor. β-Adrenergically induced relaxation [and relaxation through other receptors (43, 53)] is further mediated by cyclic nucleotides (5) such as cGMP, but especially cAMP (68), and relaxation can, therefore, be induced by the adenylyl cyclase activator forskolin (27, 57, 61).

Thus, as expected, in wild-type mice, forskolin could inhibit EFS-induced contractions, and at infinite forskolin concentrations, these contractions were fully eliminated (~1 ± 3% remaining) (Fig. 5). In contrast, as seen in Fig. 5, in ileal segments from UCP1(-/-) mice, forskolin was only able to partly inhibit the EFS-induced contractions. Even when tested in independent experiments at 100 μM forskolin concentration, full inhibition was not obtained (not shown), and the Michaelis-Menten analysis indicates that even at infinite forskolin concentrations 11 ± 3% of the contractions would be remaining (P < 0.05; n = 3). Forskolin also had a nearly threefold lower apparent affinity in these segments [IC50 was 159 ± 32 nM in intestine from UCP1(+/-) mice and 393 ± 92 nM in intestine from UCP1(-/-) mice (P < 0.05, n = 3) (Fig. 5)].

It was therefore concluded that the difference between intestine from UCP1(+/-) and UCP1(-/-) mice was not due to an alteration in β-receptor function but was located in the pathway between cAMP and muscle relaxation. Correspondingly, the presence of the UCP1-dependent relaxation was not dependent on the β-receptor itself being stimulated; rather, any receptor, the function of which is mediated via cAMP, would be expected to demonstrate an UCP1-dependent component.

DISCUSSION

In the present investigation, we have demonstrated the existence of a UCP1-dependent component in the pathway for β-adrenergically induced relaxation in intestine. Whereas the presence of this component may be an indirect consequence of the absence of UCP1 in brown adipose tissue, it is more likely that it is explainable by the absence of UCP1 in intestinal cells. Thus a
UCP1-dependent mechanism involved in the transduction pathway from β-adrenergic receptor stimulation and cAMP increases to intestinal relaxation may exist. A hypothesis for a functional role of UCP1 in contraction/relaxation control is presented below. In extension of these studies, it may be envisaged that the existence of a UCP1-dependent component in relaxation may not be restricted to adrenergic control of intestinal contraction but may be of significance for the understanding of the function of other organs apparently expressing UCP1 and exhibiting peristalsis.

β-Adrenergically Induced Relaxation Versus Other Relaxing Agents

We have here focused on the significance of UCP1 for the mediation of β-adrenergically induced relaxation in the ileal segments. As the effect was seen both when the segments were stimulated electrically and when they were directly stimulated with carbachol, it is likely that the effects observed are not localized to the nerves but rather to some of the smooth muscle cells themselves. Furthermore, because a UCP1-dependent component was also observed when we increased cAMP levels in these segments by direct stimulation of adenylyl cyclase (Fig. 5), the implication is that all intestinal relaxations mediated via an increase in cAMP, not only those induced by β-adrenergic receptors (23, 36), may have a significant UCP1-dependent fraction, including relaxations induced by VIP (50) and PACAP (28, 43).

Functional Consequences of Absence of UCP1 in the Intestine

For evident reasons, all dedicated investigations of phenotypical alterations in UCP1(−/−) mice have, as yet, been related to thermogenesis and metabolism. Data presented here demonstrate that the absence of UCP1 may affect the function of the intestine. There are no prior formal reports on such phenotypes, but an indication we have had of possible gastrointestinal effects of a lack of UCP1 is an occasional observation of a phenomenon of rectal prolapse, occurring in a few rather old UCP1(−/−) mice; we have not seen a similar phenomenon in any wild-type mice. We have also observed that the postmortem relaxation of the intestines occurs measurably slower in intestine from UCP1(−/−) mice (not detailed), and we noted during the experiments that the force of the response to EFS was variable during a longer time in intestinal segments from UCP1(−/−) mice. The significance of alterations in intestinal relaxation under normal conditions may, however, be limited. It is generally assumed that the degree of sympathetic control of the gut is normally low, and is only activated in defense reactions, i.e., during stress (69), inflammation (45, 55) and e.g., in postoperative ileus (12, 29). Manifestations of the consequences of the absence of the UCP1-dependent part of relaxation may thus be obvious only under such conditions. It is also clear from our observations that the presence of UCP1 is not an absolute necessity for the functioning of the intestine, in contrast to the absolute necessity of UCP1 for competent brown adipose tissue function (17, 21, 46, 47, 49). This is in accordance with the fact that UCP1 is not found in the genome of any nonmammalian organism. Thus the presence of UCP1-dependent relaxation is apparently a specific and additional mammalian feature, the survival value of which is presently unknown.

It may be important to consider whether the existence of the UCP1-dependent adrenergically induced intestinal relaxation demonstrated here challenges the conclusions from earlier studies on the phenotype of the UCP1(−/−) mice. However, the conclusions of studies published to date (7, 10, 17, 21, 46–49) would not seem to be influenced by this new aspect: the reported effects are adequately understood based on a functional defect in brown adipose tissue alone. However, the situation may be different regarding the analysis of so-called brown-adipose-tissue-deficient animals (13, 18, 41, 44). In these transgenic animals, diphtheria toxin is expressed under the UCP1 promotor. Thus any cell that normally expresses UCP1 will also express diphtheria toxin and this toxin will kill the cell. This means that the intestinal cells expressing UCP1 will be eliminated, which may have broader consequences than the partial deterioration of their function reported here. Thus certain phenotypic features of the so-called brown adipose tissue-deficient mice may be ascribable to e.g., intestinal effects.

Mechanism of β-Adrenergically Induced Intestinal Relaxation

The signaling system leading from acetylcholine stimulation of muscarinic receptors to contraction of...
smooth muscle cells is fairly well understood (43, 53, 65). However, the mechanism for the relaxation pathway is still unclear (73). cAMP-induced phosphorylation of specific proteins, such as telokin (42, 70), has been discussed, but the further effect of these phosphorylations is also unknown. On the basis of the experiments reported here, together with what is known about UCP1 function in brown adipose tissue (reviewed in Refs. 16, 35, 51, 60), a first, hypothetical model for a novel pathway can be formulated (Fig. 6).

In the contraction process induced by acetylcholine binding to the muscarinic receptors and intracellularly mediated via an increase in cytosolic Ca\(^{2+}\) levels, ATP is used (31, 32, 65). This ATP is of mitochondrial origin. Adrenergic stimulation through β-receptors can be expected to lead to an increase in cAMP levels, and this could subsequently lead to activation of UCP1. This would lead to mitochondrial uncoupling, i.e., some of the protons pumped out from the mitochondria by the respiratory chain would return to the mitochondrial matrix through UCP1, i.e., without passing through the ATP synthase, and less ATP would thus be formed. If this process is of sufficient magnitude, the ATP/ADP ratio in the cells will decrease, and less ATP will, therefore, be available for the contraction process. Thus a graded counteraction of induced contraction would be caused by adrenergic stimulation, although the Ca\(^{2+}\) level may be unaffected, i.e., a given level of Ca\(^{2+}\) may be less effective in inducing contraction, as has been found earlier (73). The fact that relaxation takes place, although not to the same extent, in the absence of UCP1, implies that other mechanisms are responsible for the major part of the relaxation process, but the UCP1-dependent relaxation would seem to have a higher sensitivity to β-adrenergic stimulation/cAMP levels.

In brown adipocytes, the link between increased cAMP levels and the associated increase in protein kinase A activity (14) is associated with phosphorylation (activation) of hormone-sensitive lipase (63) and perilipin (6), leading to stimulated hydrolysis of triglycerides, and the released fatty acids, either in themselves or in some metabolite form, activate UCP1 (16, 35, 51, 60). Stores of triglyceride are apparently not observable in the longitudinal muscle layer of the intestine, and there are no reports that hormone-sensitive lipase or perilipin are expressed in this layer (although this may be due to the very small fraction of total tissue that constitutes the cells of interest). In the apparent absence of the established pathway for UCP1 activation, an interesting question as to the nature of the intracellular physiological activator of UCP1 in these cells arises. However, the mitochondria containing UCP1 are only a very small fraction of all mitochondria in intestinal tissue, and to examine the function of UCP1 in intestinal mitochondrial preparations is probably unfeasible, due to high dilution by mitochondria from other parts of the tissue.

**Perspectives**

The present studies were restricted to analyzing the effect of UCP1 ablation on relaxation of ileal segments. Evidently, the question may be raised as to whether similar mechanisms are involved in the relaxation of other types of smooth muscle. Because UCP1 is not present in the smooth muscle layers of blood vessels (52), the mechanism discussed here is not relevant in the control of vessel contractility and thus not for blood pressure in general. This distinction may be paralleled by the presence of distinct forms of myosin in vascular and peristaltic smooth muscle (30, 64).

However, the results presented here may be of interest concerning the function of other organs in which UCP1 has been indicated: stomach, esophagus, ureter origin, epididymus, ductus deferens, and uterus. It is noteworthy that in most of these organs, a cAMP-mediated relaxation is seen [uterus (39, 56); vas deferens (27), urinary bladder (58), detrusor (57), distal colon (33), ileum (61), esophagus, and ureter (54)]. It is reasonable to assume that this relaxation could partly be UCP1-dependent, as demonstrated here for the ileal segments. Furthermore, it is also noteworthy that these organs all exhibit peristaltic motion. Regulated phasic contractile activity is coordinated, at least in the intestine, by the pacemaker cells, the intestinal cells of Cajal (reviewed in Refs. 62, 67), which are smooth muscle cells characterized e.g., by a high mitochondrial content (71). Similar pacemaker cells have at least been identified in the ureter (34) and discussed concerning the uterus (74). It is therefore not unlikely that UCP1 expression, cAMP-mediated relaxation, pacemaker cells, and peristalsis may be functionally linked in several organs, in addition to the intestine directly studied here.

Peristalsis, in addition to being required for the handling of food through the gastrointestinal system, is important in such functions as semen transfer, uterine function, and urine production. In the absence of dedicated observations and experiments, it may not be unexpected that no other phenotypic alterations have as yet been reported in UCP1(-/-) mice than those related to thermogenesis and now intestinal relaxation. However, the present observations may stimulate new types of dedicated studies of phenotypic manifestations of UCP1 ablation. Establishment of the significance of UCP1 in these contexts adds new challenges to the study of this unique mammalian protein.

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