

Cytokine regulation of β -adrenergic responses in airway smooth muscle

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Decreased responsiveness to β -adrenergic receptor agonists is a characteristic feature of human asthma. One explanation for this observation is that cytokines released in the asthmatic airway have direct effects on airway smooth muscle cells that reduce the ability of the cells to relax in response to β -agonists. This review summarizes data indicating that both inflammatory cytokines, such as IL-1 β and TNF- α , and Th2 cytokines, such as IL-13 and IL-5, have the capacity to decrease the ability of cultured airway smooth muscle cells to relax or to generate cyclic AMP in response to β -agonists, such as isoproterenol. These effects are observed in smooth muscle from human airways and airway smooth muscle of other species. In human airway smooth muscle, the effects of IL-1 β and TNF- α appear to be mediated through expression of cyclooxygenase-2, whereas the effect of IL-13 requires activation of the extracellular signal-regulated kinase-mitogen-activated protein kinase pathway. IL-1 β and TNF- α also inhibit the ability of β -agonists to drive airway smooth muscle gene expression through pathways dependent on cyclic AMP response elements. Understanding the mechanistic basis for the effects of these cytokines may prove to be an important step in improving the efficacy of β -agonists for the treatment of asthma. (*J Allergy Clin Immunol* 2002;110:S255-60.)

Key words: IL-1 β , TNF- α , IL-13, mitogen-activating protein kinase, cyclooxygenase-2

Decreased responsiveness to β -adrenergic receptor (β AR) agonists is a characteristic feature of asthma. Airways of patients with asthma have a reduced capacity to dilate in response to β -agonists both in vivo and in vitro.^{1,2} The mechanistic basis for these changes in responsiveness has not been established. However, the observation that animal models of asthma also exhibit decreased responses to β -agonists^{3,4} suggests that use of asthma medication does not account for these changes.

One potential explanation for the decreased β -adrenergic responsiveness observed in asthma is that it results from the effects of cytokines present in the asthmatic airway on β_2 -adrenergic signal transduction in airway smooth muscle. In support of this hypothesis, increased levels of a number of cytokines, including IL-1 β , TNF- α , IL-4, IL-5, IL-13, and IL-9, have been observed in bronchoalveolar lavage fluid from patients with symptomatic asthma. Cells in the airway wall also show increased

Abbreviations used

β AR:	β -Adrenergic receptor
cAMP:	Cyclic AMP
CBP:	Cyclic AMP response elements binding protein binding protein
COX:	Cyclooxygenase
CRE:	Cyclic AMP response elements
CREB:	Cyclic AMP response elements binding protein
ERK:	Extracellular signal-regulated kinase
HASM:	Human airway smooth muscle
MAP:	Mitogen-activated protein
NF- κ B:	Nuclear factor κ B
PGE ₂ :	Prostaglandin E ₂
PKA:	Protein kinase A

expression of these cytokines. Furthermore, many of these cytokines have been shown to reduce the capacity of airway smooth muscle to generate cyclic AMP (cAMP) or to relax in response to the β -agonist isoproterenol (Table I).⁵⁻²⁰ The purpose of this review is to summarize what is known about the effects of cytokines on β -adrenergic responsiveness of airway smooth muscle. Wherever possible, it will focus on results obtained in human cells.

EFFECTS OF IL-1 β AND TNF- α

My colleagues and I have previously reported that in cultured human airway smooth muscle (HASM) cells IL-1 β causes a marked and dose-dependent inhibition of the ability of isoproterenol to decrease cell stiffness, a proxy for force generation.^{5,6,21-23} Our results indicate that IL-1 β exerts these effects through a pathway requiring cyclooxygenase (COX)-2 expression that is dependent on activation of the extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein (MAP) kinases. Expression of COX-2 results in increased release of prostaglandin E₂ (PGE₂) from HASM cells, which leads to an increase in cAMP. Our results are consistent with the hypothesis that the increase in protein kinase A (PKA) activity that results from this increase in cAMP leads to a decrease in β -adrenergic responsiveness by phosphorylating the β_2 AR and uncoupling it from G_s.

The data supporting this hypothesis are as follows. We showed that despite potent effects on responses to isoproterenol, IL-1 β has no effect on cell stiffness responses to dibutyl cAMP, a cell-permeant analog of cAMP,⁵ indicating that the cytokine does not alter PKA expression or activity or any of the downstream effectors that

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TABLE I. Effects of cytokines on β -adrenergic responses of airway smooth muscle

Cytokine	Species	Response to isoproterenol	References
IL-1 β	Human, guinea pig, rabbit, rat	$\downarrow\downarrow\downarrow$	5-10
TNF- α	Human, guinea pig, rabbit, dog	No effect or $\downarrow\downarrow$	7, 9, 11-13
Transforming growth factor- β	Human	$\downarrow$	14
IL-13	Human, rabbit	$\downarrow$	15, 16
IL-5	Rabbit	$\downarrow$	17, 18
IL-10	Rabbit	$\downarrow$	19
GM-CSF	Rabbit	$\downarrow$	17
IL-4, IL-2, IFN- γ	Human, guinea pig, rabbit	No effect	9, 12, 15, 17, 20

Arrow, Decrease.

cause the cells to relax. IL-1 β also decreases the ability of isoproterenol to induce cAMP formation in HASM cells, but it has no effect on responses to forskolin, an agent that directly activates adenylyl cyclase. These results suggest that the effect of IL-1 β lies upstream of adenylyl cyclase. Because our data indicated that β_2 AR expression and G_s expression are unaffected by IL-1 β , we reasoned that IL-1 β was acting at the level of β AR- G_s coupling.

We subsequently demonstrated that IL-1 β markedly up-regulates COX-2 expression and PGE₂ release, that the effect of IL-1 β on responses to isoproterenol is abolished in HASM cells that are treated with COX-2 inhibitors, and that exogenous PGE₂ mimics the effect of IL-1 β .⁶ Similar results have been obtained by other investigators.^{7,8} Consistent with a role for COX-2 in these events, our results indicated that the effect of IL-1 β required at least 6 hours for its induction and could be abolished by a protein synthesis inhibitor. Because IL-1 β by itself caused a small but significant increase in cAMP formation that was abolished by COX-2 inhibitors, we reasoned that the role of COX-2 was to increase PGE₂ release, resulting in increased cAMP formation. We further reasoned that the increased PKA activity resulting from this increase in cAMP could be causing the decrease in β -adrenergic responsiveness by phosphorylating the β AR and uncoupling it from G_s (Fig 1).^{24,25} Further investigations indicated that the effect of IL-1 β was dependent on activation of the ERK and p38 MAP kinase pathways.^{21,22} Furthermore, PD98059 and U0126 (inhibitors of MAP kinase kinase, the enzyme upstream of ERK), and SB203580 (an inhibitor of p38), each blocked the changes in isoproterenol-induced cell stiffness that were elicited by IL-1 β . It is likely that the role of these MAP kinases is to induce COX-2 expression, because U0126, PD98059, and SB203580 also substantially inhibit IL-1 β -induced COX-2 expression and PGE₂ release. The effect of IL-1 β on β -adrenergic responsiveness in HASM is also abolished by glucocorticoids, an effect that is probably related to the ability of these agents to inhibit IL-1 β -induced COX-2 expression.²³

COX-2 has been implicated in the effects of IL-1 β on β -adrenergic responses in HASM cells, and the COX-2 promoter contains 2 putative binding sites for the transcription factor nuclear factor κ B (NF- κ B) that have been demonstrated to be important in COX-2 expression in

other cell types. We therefore examined the role of NF- κ B in IL-1 β -induced COX-2 expression in HASM cells. There was a robust increase in binding to NF- κ B consensus sequences of proteins from nuclear extracts of IL-1 β -treated cells relative to control HASM cells.²³ IL-1 β also caused a robust increase in luciferase activity in HASM cells transfected with a construct consisting of a luciferase gene driven by a promoter consisting of multiple NF- κ B elements²² that was blocked by the proteasome inhibitor MG132. However, MG132 did not prevent the induction of COX-2 expression by IL-1 β , suggesting that NF- κ B is not important in the induction of COX-2 by IL-1 β .²²

In contrast to the effects of IL-1 β , we did not observe any substantial effect of TNF- α on β -adrenergic responsiveness in HASM cells.¹¹ Similar results have been obtained by others.⁷ When we administered IL-1 β and TNF- α to HASM cells simultaneously, however, at concentrations that on their own were ineffective, we observed a marked suppression of isoproterenol-induced changes in cell stiffness. The same concentrations of IL-1 β and TNF- α also acted synergistically in the induction of COX-2 expression and PGE₂ release through effects on COX-2 promoter activity. Furthermore, the relatively selective COX-2 inhibitor NS-398 abolished the effects of combined treatment with IL-1 β and TNF- α , suggesting that COX-2-generated prostanoids mediate these effects on β -adrenergic responsiveness. These results may be particularly important because they suggest that IL-1 β and TNF- α , at concentrations present in bronchoalveolar lavage fluid from patients with asthma, can have important functional consequences if they are released simultaneously.

A decrease in β -adrenergic relaxation of airway smooth muscle caused by IL-1 β or TNF- α has also been observed in other species, including guinea pigs, rats, rabbits, and dogs.^{9,10,12,13} As was the case in HASM, in all these species except the rat the effects of IL-1 β and TNF- α did not involve changes in β_2 AR expression. However, in these species, the effects of IL-1 β or TNF- α appear to be mediated not through a COX-2-dependent pathway, as occurs in HASM, but rather through events involving G_i . There were some major differences in the protocols used in the human studies versus those used in other species that may explain this apparent discrepancy. In studies examining the effects of IL-1 β or TNF- α in

nonhuman species, responses to β -agonists were for the most part measured in whole-airway preparations that were precontracted with cholinergic agonists. It was observed that methoctramine, an M_2 -muscarinic receptor antagonist, and pertussis toxin, which adenosine diphosphate ribosylates and thereby inactivates G_i , blocked the effect of IL-1 β on rabbit tracheal relaxation. These findings suggest that the cytokine's effect was mediated through enhanced M_2 receptor- G_i -protein-coupled inhibition of adenylyl cyclase in these tissues.¹² In contrast, in HASM the responses to β -agonists were assessed in cultured cells without precontraction of the smooth muscle, that is, in a situation in which G_i was not activated.⁵

EFFECTS OF Th2 CYTOKINES

One of the major factors distinguishing the asthmatic condition is the expression in the airways of cytokines derived from Th2 lymphocytes, such as IL-4 and IL-13. We therefore sought to determine the effects of IL-4 and IL-13 on β -adrenergic responsiveness in HASM cells.¹⁵ Our results indicate that IL-13 decreases isoproterenol-induced changes in cell stiffness, although the effect is not as marked as that observed with IL-1 β . In contrast, IL-13 has no effect on cell stiffness responses to dibutyl cAMP. These results suggest that IL-13 does not alter either the ability of cAMP to activate PKA or the targets of PKA that ultimately mediate cell relaxation. The results also suggest that the effect of IL-13 on cell stiffness responses to isoproterenol is unlikely to be related to nonspecific effects on the ability of the cells to relax. In addition to effects on cell stiffness, IL-13 also attenuates isoproterenol-induced cAMP formation in HASM cells. Because the data were gathered in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine, IL-13-induced changes in cAMP degradation are unlikely to have contributed to these responses. The observation that cAMP formation stimulated by forskolin, which directly activates adenylyl cyclase, is not influenced by IL-13 suggests that the effect of IL-13 is likely to be upstream of cyclase activation, at the level of the β AR, G_s , or their coupling. Because IL-13 activates ERK in HASM cells and because inhibition of ERK with U0126 restores the ability of isoproterenol to decrease cell stiffness in IL-13-treated cells, it is likely that IL-13 induces its effects on β -adrenergic responsiveness through an ERK-dependent pathway.

In contrast to the effects of IL-13, we were unable to observe any effect of IL-4 on β -adrenergic responsiveness in HASM cells, despite the fact that IL-13 and IL-4 use the same receptor for signaling in these cells.

Other investigators have also reported effects of Th2 cytokines on β -adrenergic responsiveness of airway smooth muscle, albeit in other species. Hakonarson et al^{17,18} reported that IL-5 decreased the ability of isoproterenol to cause relaxation of cholinergically contracted rabbit airways in tissue baths. On the basis of their observations that IL-1 β was released after stimulation with IL-5, that IL-1 β also caused β -adrenergic desensitization,

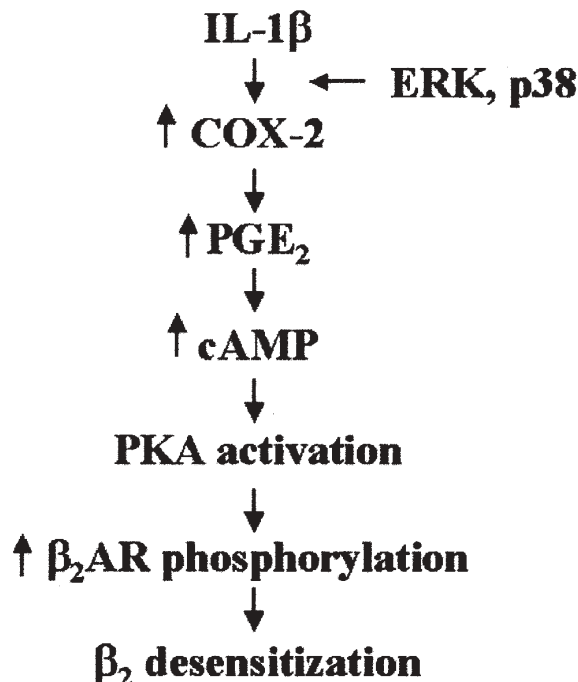


FIG 1. Proposed mechanism of action of IL-1 β on β -adrenergic responsiveness in cultured HASM cells. (From Laporte JD, Moore PE, Panettieri RA, Moeller W, Heyder J, Shore SA. Prostanoids mediate IL-1 β -induced β -adrenergic hyporesponsiveness in human airway smooth muscle cells. *Am J Physiol* 1998;275:L491-501. With permission.)

and that an IL-1 receptor antagonist blocked the effects of IL-5, the authors concluded that the effect of IL-5 was mediated through release of IL-1 β . The authors subsequently demonstrated that exogenous IL-13 and IL-10, but not IL-4, inhibited isoproterenol-induced relaxation of rabbit airways.^{16,19} Because the effects of these cytokines could be ablated with an IL-5 antibody and because both IL-13 and IL-10 were able to induce release of IL-5 from airway smooth muscle, the authors concluded that the effects of these two cytokines were mediated through release of IL-5 and its subsequent ability to release IL-1 β . Taken together, the results suggest that airway smooth muscle is an important target for Th2 cytokines. In contrast, the Th1 cytokine IFN γ appears either to have no effect or to increase sensitivity to β -agonists in airway smooth muscle.^{17,20}

GENE EXPRESSION MEDIATED BY CAMP RESPONSE ELEMENTS

In addition to relaxing HASM cells, β -agonist activation of PKA induces changes in the transcription of genes that contain cAMP response elements (CREs) in their promoter regions (Fig 2). Activation of CREs by β -agonists occurs through PKA phosphorylation of CRE binding protein (CREB) at serine 133.²⁶ Phosphorylation of CREB permits its interaction with CREB-binding protein (CBP) and p300, which interact with the basal transcriptional apparatus to initiate transcription.

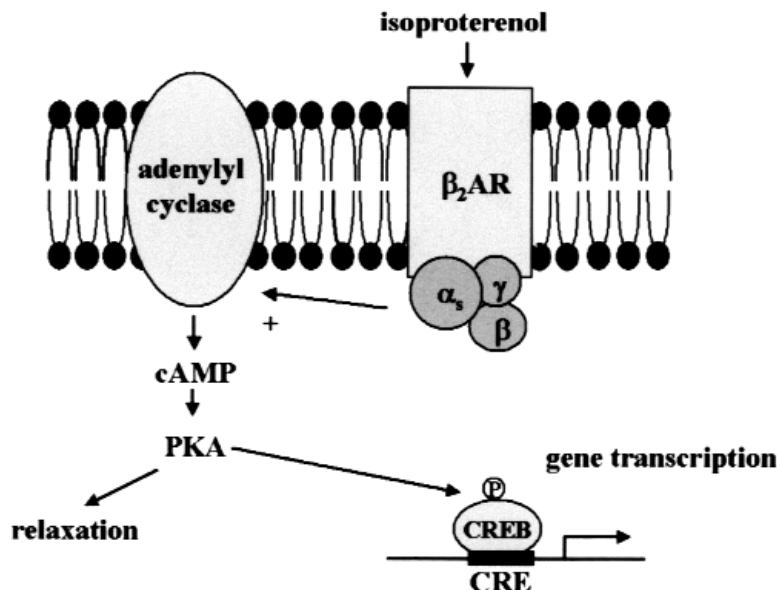


FIG 2. Mechanism of action of isoproterenol on airway smooth muscle relaxation and gene expression.

We sought to determine whether IL-1 β in addition to its ability to inhibit β -agonist-induced relaxation of smooth muscle, also inhibits β -agonist-induced CRE-driven gene expression. In isoproterenol-treated cells we observed a substantial increase in CREB phosphorylation relative to control cells, an increase that was largely suppressed when cells were pretreated with IL-1 β . Isoproterenol also caused a marked increase in luciferase expression in HASM cells transiently transfected with a luciferase reporter construct driven by multiple CREs, and this increase in luciferase activity was greatly diminished in cells treated with IL-1 β before addition of isoproterenol. Indomethacin, U0126, and dexamethasone were only partially effective in restoring the ability of isoproterenol to induce CRE-driven gene expression in IL-1 β -treated cells despite the almost complete restoration of isoproterenol-induced cAMP formation or cell stiffness changes induced by each of these compounds. IL-1 β also suppressed the ability of dibutyl cAMP to induce CRE-driven gene expression. Taken in conjunction with the observation that IL-1 β does not alter the activity of PKA in HASM cells,⁵ the results suggest that the effect of IL-1 β on isoproterenol-induced CRE-driven gene expression is the result of its influence on events downstream of PKA activation.

Because interaction of CBP with phosphorylated CREB is vital to the formation of the basal transcriptional apparatus that allows transcription of CRE-dependent genes,²⁶ it is possible that the ability of IL-1 β to attenuate isoproterenol-induced CRE-driven gene expression is the result of effects of the cytokine on recruitment of CBP to phosphorylated CREB. For example, CBP interacts with transcriptional factors other than CREB, at least some of

which are activated by IL-1 β , and there is evidence that nuclear competition between transcription factors for limiting amounts of CBP results in mutual repression of the ability of these factors to activate transcription.²⁷

CONCLUSIONS

The data presented here indicate that both inflammatory cytokines, such as IL-1 β and TNF- α , and Th2 cytokines, such as IL-13 and IL-5, have the capacity to reduce the ability of airway smooth muscle to relax in response to β -agonists and to inhibit the ability of β -agonists to drive airway smooth muscle gene expression through CRE-dependent pathways. Taken in conjunction with the observations that these cytokines are present in increased amounts in the microenvironment surrounding airway smooth muscle in patients with asthma, these results suggest that cytokines may contribute to the β -adrenergic hyporesponsiveness that is characteristic of asthma. If so, understanding the mechanistic basis for the effects of these cytokines may prove to be an important step in improving the efficacy of β -agonists for the treatment of asthma.

DISCUSSION SESSION

Question: If you block COX-2 instead of stimulating its production, which promotes relaxation, with a nonspecific inhibitor such as indomethacin or a nonsteroidal anti-inflammatory drug, does that augment the contractile response or inhibit the relaxing response?

Dr Shore: We haven't yet studied the impact of COX-2 inhibitors on contractile responses. COX-2 inhibitors on their own have no effect on relaxant responses to β -agonists, but they inhibit the reduction in β -agonist responses that are induced by IL-1 β .

Question: Is phosphodiesterase involved in desensitization?

Dr Shore: I do not think that phosphodiesterase is important.

Whenever we did cAMP measurements, which were affected by IL-1 β , we always included a phosphodiesterase inhibitor with our cells to prevent the phosphodiesterases from breaking down the cAMP. It could be that there is an additional effect of IL-1 and phosphodiesterase, but if so it is not the whole story, because you can see it even in the presence of phosphodiesterase inhibitors.

Question: There are data in the literature about synergy between IL-13, IL-4, and TNF and IL-1. Have you used a combination of the TNF or IL-1 with IL-13 or IL-4?

Dr Shore: Yes, we have done that with IL-1 and TNF, and in fact we do see synergy. We used a concentration of IL-1 that caused a small effect and added IL-13. We saw a loss of the ability of the cells to bronchodilate. IL-4 does not do anything, and I really do not know why. It is interesting because the IL-4 and IL-13 are acting on the same receptor in these cells. In many other cell types, there is a γ chain that can interact with the IL-4 receptor to form dimers and produce effects that way. The other way that IL-4 works is it forms dimers with the IL-13 receptor. Our cells do not make the γ chain, as we have shown by polymerase chain reaction. So they both have to be acting by the same dimer of the IL-13, IL-4 receptor, and yet they are producing different effects.

Question: Do COX-2 inhibitors have any effect on the bronchodilatory effects of β -agonists in patients with asthma?

Dr Shore: To my knowledge there has never been a trial combining aspirin or a similar compound with bronchodilators. This would be a complicated experimental design, because COX mediators have so many effects that it would be difficult to focus on the bronchodilatory effect. On the other hand, now that we have COX-2 inhibitors, which may be safer for patients with asthma than aspirin, it might be possible to perform this study.

Question: Regarding the role of PGE₂ in receptor desensitization, have you studied this in cells other than smooth muscle?

Dr Shore: We have not studied this in any cell type other than the smooth muscle cell. I imagine the reports would be similar in almost any cell. PGE₂ will activate PKA, and PKA will phosphorylate β -receptors in virtually any cell type. However, I have no data to support this.

Question: I am intrigued with the notion that a small amount of PKA stimulation by PGE₂ is enough to down-regulate the ability to achieve a great degree of stimulation with the β_2 -adrenergic receptor. Is it reasonable to infer from this that a small amount of β -adrenergic stimulation with a β -agonist might have the same influence, so that low-dose treatment with inhaled β -agonists might be detrimental and higher doses might be beneficial?

Dr Shore: It is known that a small dose of β -agonist in these cells will down-regulate the response to isoproterenol. After even an hour of treatment with 10⁻⁷ or 10⁻⁸ mol/L isoproterenol, we see down-regulation of the response by approximately 35%. If the exposure is increased to 24 hours, there is a 75% reduction in response. Clearly, it does not take a lot of cAMP formation to down-regulate the receptors. I am uncertain whether that means that a low-dose β -agonist is detrimental.

Question: There are multiple PGE₂ receptor subtypes. Can these pathways be excluded as a source of the effect rather than a G_s heterologous desensitization?

Dr Shore: I do not have the data to exclude that possibility. The effect is produced by something that blocks COX-2. I am assuming that PGE₂ is involved because of the effect of the small increase in cAMP that we observed.

Question: What is the relaxant effect of the IL-1 in the cells?

Dr Shore: By itself, it has no stiffness, probably because the amount of cAMP that gets produced is fairly small, about 30%. The highest concentration of IL-1 that we used was 20 ng/mL. That is what happens at 20 ng/mL. The effect is seen even at concentrations as low as 2 ng/mL of IL-1.

Question: Did you compare the time course of the cAMP response to IL-1 with the time course in response to β -agonists?

Dr Shore: We did not study the time course for cAMP, only for stiffness.

Comment: Somebody asked about the specificity of this effect of IL-1 β on smooth muscle. We have looked at the effect of IL-1 β on cultured human airway epithelial cells. There are some differences compared with the smooth muscle. You see desensitization in response to isoproterenol, but it appears to be an effect of increased expression of G_{ai}. G_{ai} is induced, and you can block that phenomenon with pertussis toxin. The other interesting difference between the epithelial cells and the smooth muscle cells is that IL-1 has profound effects on β_2 gene expression. There is a marked up-regulation of receptor protein and message by 3- to 5-fold. Clearly, cell-type differences are important. We did not see an effect with pertussis toxin. We observed no effect on the β_2 -receptor expression itself and no change in promoter activity.

Question: Does acetylcholine down-regulate β -adrenergic receptors?

Dr Shore: I am not certain. Hall has done a similar experiment with acetylcholine. There are no M3 receptors in our smooth muscle cells once they are cultured. Hall was activating M2 receptors, and he was able to inhibit isoproterenol-induced cAMP formation by the M2 receptors, presumably through a G_i-mediated effect, because it was blocked by pertussis toxin.

Question: Are your smooth muscle cells in vitro precontracted?

Dr Shore: No. When we cultured the smooth muscle cells on plastic dishes, they seemed to have some kind of endogenous tone. We do not know what causes this. The only thing that blocks this is isoproterenol.

Question: In your experiments, were the cells serum starved?

Dr Shore: Yes, all the cells were serum starved for at least 24 hours.

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