Potentiation of A₂ Purinoceptor-Stimulated Surfactant Phospholipid Secretion in Primary Cultures of Rat Type II Pneumocytes

Matthias Griese,* Laurice I. Gobran, and Seamus A. Rooney

Division of Perinatal Medicine, Department of Pediatrics, Yale University School of Medicine, P. O. Box 3333, 333 Cedar Street, New Haven, CT 06510, USA

Abstract. Surfactant secretion is mediated by a number of different signal-transduction mechanisms. Positive and negative interactions between different signaling pathways can have an important influence on the overall regulation of secretion. To examine interactions between the adenosine A₂ receptor-mediated pathway and those involving activation of protein kinase C and a Ca⁺⁺/calmodulin-dependent system, we examined the effect of the A₂ agonist 5'-N(ethylcarboxyamido) adenosine (NECA) in combination with 2 activators of protein kinase C, 12-O-tetradecanoylphorbol-13-acetate (TPA) and dioctanoylglycerol, and the Ca⁺⁺ ionophore ionomycin on phosphatidylcholine secretion in primary cultures of rat type II cells. The individual agonists increased secretion 3–5-fold over the rate in control cells. The stimulatory effects of NECA + TPA, NECA + dioctanoylglycerol, and NECA + ionomycin were 44%, 20%, and 44% greater, respectively, than expected by addition of the effects of the individual agonists. NECA increased cAMP formation while the other agonists did not. However, the effect of NECA on cAMP formation was significantly enhanced by TPA and dioctanoylglycerol, while the duration of the increase in cAMP level was prolonged by dioctanoylglycerol and ionomycin. Although the possible involvement of other second messenger systems cannot be excluded, we speculate that the synergistic interaction between the agonists in stimulating phosphatidylcholine secretion is mediated by increased cAMP levels.

Key words: Pulmonary surfactant—Phosphatidylcholine secretion—Adenosine A₂ receptor—5'-N(ethylcarboxyamido) adenosine (NECA)—12-O-tetradecanoylphorbol-13-acetate—Protein kinase C inhibitors.

* Present address: Ludwig-Maximilians-Universität, Kinderpoliklinik, Munich, Germany
Offprint requests to: S. A. Rooney
Introduction

Secretion of phosphatidylcholine, the major surfactant lipid, can be stimulated by several physiological and pharmacologic agents [4, 25, 36]. Known surfactant secretagogues in isolated type II cells include β-adrenergic agonists, P₁ (adenosine) and P₂ (ATP) purinoceptor agonists, the protein kinase C activators 12-O-tetradecanoylphorbol-13-acetate (TPA) and diacylglycerols, the ionophores ionomycin and A23187, which increase intracellular Ca²⁺ levels, as well as other agents [4, 25, 36].

The effects of the different surfactant secretagogues are mediated by a number of signal-transduction mechanisms. Activation of β-adrenergic and adenosine A₂ receptors leads to formation of cyclic AMP (cAMP) and ultimate activation of cAMP-dependent protein kinase [8, 20]. There is evidence that the stimulatory effect of ATP is mediated by both P₁ receptors coupled to the adenylate cyclase system [13, 33] and P₂ receptors coupled to phosphoinositide-specific phospholipase C, activation of which leads to formation of the second messengers inositol trisphosphate (IP₃) and diacylglycerols [12, 21, 33]. These second messengers in turn promote mobilization of intracellular Ca²⁺ and activation of protein kinase C [21]. In many systems activation of protein kinase C is further coupled to a phospholipase D acting on phosphatidylcholine or phosphatidylethanolamine [2, 17] and there is recent evidence that ATP and TPA activate phospholipase D in the type II cell [3, 26]. Evidence for involvement of a Ca²⁺/calmodulin-dependent protein kinase or another calmodulin-dependent step is suggested by the findings that the stimulatory effects of ionomycin [24] and ATP [24, 32] on phosphatidylcholine secretion were antagonized by calmodulin antagonists.

Positive and negative interactions between the different signaling pathways are likely to play an important role in the overall physiological regulation of surfactant secretion. Information on interactions between different secretagogues can also help to elucidate the signal-transduction pathways involved. We previously examined interactions between purinoceptor agonists and other surfactant secretagogues in type II cells both on the functional and second messenger levels. Such studies revealed that although the effects of ATP and TPA on secretion were additive, suggesting independent signaling mechanisms, pretreatment of the cells with TPA led to a reduction in the subsequent stimulatory effect of ATP on both secretion and IP₃ formation [12]. The stimulatory effect of ATP on secretion was less than additive to that of the adenosine A₂ receptor agonist 5′-(N-ethylcarboxyamido)adenosine (NECA), suggesting an overlap between the signaling pathways mediating the effects of those 2 purinoceptor agonists [13]. Finally, in keeping with a common mechanism of action of adenosine receptor and β-receptor agonists, the combination of terbutaline with adenosine, AMP or NECA was no more stimulatory than the individual agonists alone [8].

The objective of the present study was to examine interactions between the adenosine A₂ receptor signaling system and signal-transduction mechanisms involving activation of protein kinase C and Ca²⁺/calmodulin-dependent steps. We therefore examined the influence of NECA in combination with TPA, a