REGULATION BY β-ADRENERGIC RECEPTOR AND MUSCARINIC CHOLINERGIC RECEPTOR ACTIVATION OF INTRACELLULAR CYCLIC AMP AND CYCLIC GMP LEVELS IN RAT LUNG SLICES*

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Summary

The effects of adrenergic and cholinergic agents, present singly or in combination, on the levels of cyclic AMP and cyclic GMP in slices of rat lung were studied. It was found that isoproterenol increased pulmonary cyclic AMP levels about 3-fold, and this increase was abolished by propranolol, but not by phenoxybenzamine. Acetylcholine increased the cyclic GMP levels also about 3-fold (thus raising its tissue content above that of cyclic AMP), and this increment was largely reduced by atropine, but not by hexamethonium. While without effects on the cyclic GMP levels when present alone, isoproterenol antagonized acetylcholine in increasing cyclic GMP levels. Acetylcholine, while lacking effects on the basal levels of cyclic AMP, on the other hand, depressed the augmented levels caused by isoproterenol.

The data presented indicate that cyclic GMP may mediate the cholinergic action in lung and that the pulmonary cyclic GMP levels are also closely regulated by β-adrenergic receptor activation.

Recent studies indicate that cholinergic agonists increase cyclic GMP levels, with little or no effects on the basal levels of cyclic AMP, in heart (1,2), intestinal smooth muscle (2), and brain (2), and that a mutual antagonism appears to exist between the activation of β-adrenergic and muscarinic cholinergic receptors in terms of their abilities to regulate the intracellular levels of these two cyclic nucleotides (1,2).

Compared to many other biological materials so far examined, lung is one of the few tissues that has been shown to have an exceptionally high guanylate cyclase activity (3) and cyclic GMP content (4,5). These findings tend to suggest an especially prominent role for cyclic GMP in lung function and metabolism. The

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TABLE I. Regulation by adrenergic agonist and antagonists of intracellular cyclic AMP and cyclic GMP levels in rat lung slices

Addition	Cyclic AMP* (pmoles/mg protein)	Cyclic GMP* (pmoles/mg protein)	Cyclic AMP to cyclic GMP ratio
None (control)	6.4 ± 0.3	4.8 ± 0.4	1.3
Isoproterenol, 1 μM	21.6 ± 1.5	4.1 ± 0.2	5.3
Isoproterenol, 1 μM + propranolol, 1 μM	9.5 ± 0.9	4.8 ± 1.0	2.0
Isoproterenol, 1 μM + phenoxybenzamine, 5 μM	20.8 ± 1.2	5.0 ± 0.6	4.2
Propranolol, 1 μM	6.1 ± 0.8	4.6 ± 0.5	1.3
Phenoxybenzamine, 5 μM	6.3 ± 0.5	4.9 ± 0.6	1.3

*Each value represents mean ± S. E. of triplicate incubations.

data presented in this communication show that the cyclic AMP and cyclic GMP levels in lung tissue are regulated in part by the adrenergic and cholinergic mechanisms similar to those shown earlier for certain tissues mentioned above.

Materials and Methods

Three to six Sprague-Dawley rats, each weighing about 160 g, were killed by decapitation and the lungs were quickly excised and placed in Krebs-Ringer bicarbonate medium (room temperature), the medium being previously gassed with 5% CO_2-95% O_2 (v/v). The lungs were first sliced (0.5 mm thick) with a Stadie-Riggs tissue slicer and were further diced into 0.5 mm cubes, using a McIlwain tissue chopper. The resulting tissue slices were then pooled, were suspended in the medium, and were washed three times with the medium. The slices were finally transferred to an appropriate volume of the fresh medium containing 3 mM theophylline and were stirred constantly to yield a near-homogeneous suspension. One-ml aliquots of the suspension (containing about 20-25 mg protein) were transferred to the incubation tubes containing the agents whose actions were to be studied. The

TABLE II. Regulation by cholinergic agonist and antagonists of intracellular cyclic AMP and cyclic GMP levels in rat lung slices

Addition	Cyclic AMP* (pmoles/mg protein)	Cyclic GMP* (pmoles/mg protein)	Cyclic AMP to cyclic GMP ratio
None (control)	5.8 ± 0.4	4.2 ± 0.2	1.4
Acetylcholine, 1 μM	5.2 ± 0.6	12.5 ± 0.5	0.4
Atropine, 1 μM	5.5 ± 0.2	4.1 ± 0.3	1.3
Acetylcholine, 1 μM + atropine, 1 μM	5.7 ± 0.2	5.1 ± 0.6	1.1
Hexamethonium, 10 μM	5.1 ± 0.6	4.0 ± 0.5	1.3
Acetylcholine, 1 μM + hexamethonium, 10 μM	5.8 ± 0.3	11.8 ± 0.6	0.5

*Each value represents mean ± S. E. of triplicate incubations.

tissue was incubated, with shaking, for 2 min at 37°. At the end of the incubation period, the medium was rapidly removed by centrifugation and aspiration, 1 ml of ice-cold 5% tricholoracetic acid was added to the tubes, and the lung tissue was homogenized directly in the incubation tubes fitted with ground glass pestles.

The amounts of cyclic AMP (6) and cyclic GMP (5) in the tissue extracts were assayed by the protein kinase catalytic methods as described earlier. Protein in the trichloracetic acid precipitates was measured by the method of Lowry et al (7).

Results and Discussion

Isoproterenol increased cyclic AMP levels more than three-fold in slices of whole lung of rat (Table 1). This increment in the pulmonary cyclic AMP content was abolished by an equal concentration (1 μM) of propranolol (a β-adrenergic receptor blocking agent), but it was not affected by phenoxybenzamine (an α-adrenergic receptor blocker) even at a higher concentration (5 μM). Isoproterenol has also been shown to increase cyclic AMP levels in human lung (8). Neither of the above agents had any significant effects on the basal levels of pulmonary cyclic GMP.

TABLE III. Reciprocal regulation by isoproterenol and acetylcholine of intracellular cyclic AMP and cyclic GMP levels in rat lung slices

Addition	Cyclic AMP* (pmoles/mg protein)	Cyclic GMP* (pmoles/mg protein)	Cyclic AMP to cyclic GMP ratio
None (control)	6.2 ± 0.4	5.1 ± 0.2	1.2
Isoproterenol, 0.1 μM	12.5 ± 0.8	5.2 ± 0.4	2.4
Isoproterenol, 1 μM	19.6 ± 0.8	4.6 ± 0.2	4.3
Acetylcholine, 0.1 μM	6.0 ± 0.5	12.6 ± 0.2	0.5
Acetylcholine, 1 μM	5.8 ± 0.4	18.2 ± 0.6	0.3
Isoproterenol, 0.1 μM, + acetylcholine, 0.1 μM	10.5 ± 0.2	9.1 ± 0.2	1.2
Isoproterenol, 0.1 μM, + acetylcholine, 1 μM	5.8 ± 0.3	12.5 ± 1.2	0.5
Isoproterenol, 1 μM + acetylcholine, 0.1 μM	14.2 ± 0.2	4.9 ± 0.2	2.9
Isoproterenol, 1 μM + acetylcholine, 1 μM	7.5 ± 0.6	8.2 ± 0.5	0.9
Isoproterenol, 1 μM + acetylcholine, 1 μM, + propranolol 1 μM	7.0 ± 0.8	19.2 ± 0.6	0.4
Isoproterenol, 1 μM + acetylcholine, 1 μM, + atropine, 1 μM	18.2 ± 1.2	6.2 ± 1.1	2.9

*Each value represents mean ± S. E. of triplicate incubations.

Acetylcholine elevated the pulmonary cyclic GMP content also about three-fold under the same incubation conditions (Table 2). The effect of the cholinergic agonist was abolished by an equal concentration (1 μM) of atropine (a muscarinic cholinergic receptor antagonist) whereas hexamethonium (a nicotinic cholinergic receptor antagonist) was without effect even at a much higher concentration (10 μM), indicating that the increases in cyclic GMP levels in lung tissue are closely regulated by muscarinic cholinergic receptor activation. Conclusions similar to that obtained with present lung studies were also obtained earlier with heart,

brain and intestinal smooth muscle (2). The basal levels of pulmonary cyclic AMP, however, was not affected by any of the above agents.

It has been shown for the first time in the present studies with lung tissues that the tissue level of cyclic GMP were found to exceed those of cyclic AMP. Thus acetylcholine augmented cyclic GMP levels to such an extent that the value of cyclic AMP to cyclic GMP ratio reached as low as 0.4 (Table 2). This was possible because lung, compared to most other tissues, not only contains the highest basal cyclic GMP concentration but also the lowest ratio of cyclic AMP to cyclic GMP content (Ref. 4, 5 and Table 2).

If one assumes that cyclic GMP-dependent protein kinase is the receptor enzyme for cyclic GMP, as shown earlier for arthropod tissues (9), it is conceivable that, in view of the high content of cyclic GMP in lung, one would be likely to detect an abundant occurrence of protein kinase specifically activated by cyclic GMP in this tissue. However, only trace amounts of cyclic GMP-dependent protein kinase, in contrast to cyclic AMP-dependent class of enzyme, have been detected. The question as to the exact mechanism of action of cyclic GMP, or the role of cyclic GMP in mammalian tissues in general, still remains to be answered.

Acetylcholine causes contraction whereas isoproterenol causes relaxation of bronchial smooth muscle. It is interesting, in view of the physiological antagonism of these agents, that an antagonism between the actions of the cholinergic and adrenergic agonists in modulating the cyclic GMP and cyclic AMP levels was also noted in the lung tissue (Table 3). The antagonism between these two groups of agents was dose-dependent. Moreover, the antagonistic action of isoproterenol on acetylcholine in its ability to increase cyclic GMP levels was blocked by propranolol, whereas the antagonistic action of acetylcholine on isoproterenol in its ability to increase tissue levels of cyclic AMP was blocked by atropine. The actions of these agents on lung slices were also explored by employing the prelabeling technique that allows one to measure the relative levels of radioactive cyclic GMP in lung tissue previously labelled with ^{3}H-guanine (10). The results obtained with these two independent methods are comparable.

It should be emphasized that lung is a heterogeneous tissue consisting of many different cell types. It is possible, therefore, that the actions of cholinergic and adrenergic receptor agonists on the pulmonary cyclic nucleotide levels may not totally reflect the responses of the smooth muscle constituent of lung. Nonetheless, the lung slice preparation described herein may be used conveniently for studying certain of the metabolic and functional properties of lung.

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