

Bronchodilator drug efficacy via cyclic AMP¹

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Duncan, P. E., Griffin, J. P., and Solomon, S. S. (1975). Thorax, 30, 192–196. Bronchodilator drug efficacy via cyclic AMP. Cyclic adenosine 3', 5'-monophosphate (cyclic AMP) as measured by radioimmunoassay is found in diced rat lung in an amount approximating one picomole per milligram of wet weight lung tissue. Incubation of rat lung with adrenaline, a beta adrenergic agent, produced a rapid increase in cyclic AMP, 100% increase at 15 seconds and 340% at 2 minutes. Isoprenaline was more stimulatory than adrenaline; noradrenaline was less stimulatory, and ephedrine produced a negligible effect. The methylxanthines, caffeine and theophylline, produced an increase in cyclic AMP concentration. Of these, caffeine was more potent, and synergism with adrenaline was demonstrated. The beta adrenergic blocking agent, propranolol, completely inhibited the expected rise in cyclic AMP secondary to adrenaline stimulation. In contrast, the alpha blocker, phentolamine, produced no effect.

This animal model offers evidence that adrenergic agents and methylxanthines act to increase cyclic AMP in lung tissue. It is likely that many of the beneficial effects of these drugs in pulmonary patients occur through similar changes and modulation of the cyclic AMP system.

The compound cyclic adenosine 3', 5'-monophosphate (cyclic AMP) has been established as a mediator substance in the mechanisms of both hormone action and release (Sutherland, 1972). Despite an extensive general literature on cyclic AMP there is only limited information available concerning this mediator in the lung (Sutherland, Robison, and Butcher, 1968; Kaliner *et al.*, 1971; Orange *et al.*, 1971; Palmer, 1971). Herein we report studies of cyclic AMP formation in a preparation of diced rat lung. Data are presented illustrating the results of classical manipulations of the second messenger system in rat lung. Studies are also presented on the relative potency of various adrenergic agents and on the results of alpha and beta adrenergic blockade on catecholamine-induced stimulation of cyclic AMP.

MATERIALS AND METHODS

Male Holtzman rats weighing 150–200 g were fed ad libitum up to the time of sacrifice. At such time

the lungs were rapidly removed, washed, and preserved in iced isotonic saline. Peripheral portions of the lungs were cut into small sections, weighed, and transferred to incubation vials.

Bovine plasma albumin V (Lot No. 30308) was obtained from Armour Pharmaceutical Company, Chicago, Illinois. Adenosine 3', 5' cyclic monophosphoric acid (Lot Nos. 109B732 and 109B163), DL-adrenaline hydrochloride, DL-isoprenaline hydrochloride, DL-noradrenaline hydrochloride, caffeine, and theophylline were obtained from Sigma Chemical Company, St. Louis, Missouri. Ephedrine sulphate (Lilly), propranolol hydrochloride (Inderal, Ayerst), and phentolamine hydrochloride (Rogitine, Ciba) were obtained from the manufacturers. Other materials included ¹²⁵I succinyl cyclic AMP tyrosine methyl ester, rabbit anti-cyclic AMP antiserum, and sheep antirabbit IgG precipitating antibody. These materials were procured from Collaborative Biomedical Products, Waltham, Mass.

At the time of experimentation 40 mg of tissue was removed from the iced saline and added to 2 ml of Krebs-Ringer-bicarbonate-4% albumin buffer (KRB) pH 7.4. Following an initial incubation (37°C) of 15 minutes the incubation mixture was decanted and replaced with fresh buffer containing various concentrations of test substance. The incubation was

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continued for 15 minutes and terminated by boiling the tissue in sodium acetate buffer (pH 6.2) for 15 minutes. Following boiling, the tissue was sonicated, and an aliquot of the extracting solution was assayed for cyclic AMP by the double immunoassay method of Steiner, Parker, and Kipnis (1970). A standard curve for each experiment was determined relating precipitable counts of immunoreactive material to concentration of cyclic AMP in picomoles. Cyclic AMP phosphodiesterase was prepared from rat brain and incubated with the diced lung preparation for 5 minutes (Solomon, 1972).

RESULTS

The Table illustrates the recovery of cyclic AMP in rat lung tissue. Rat lung contains rather less than one picomole of cyclic AMP per milligram of wet tissue. Thus 86 picomoles of cyclic AMP were recovered from 100 mg of lung. The addition of 10 picomoles exogenous cyclic AMP to a 20 mg tissue specimen yielded 30 picomoles of cyclic AMP. The addition of 0.05 ml of the degrading enzyme, phosphodiesterase, to 100 picomoles cyclic AMP resulted in 95% destruction of the substrate in 15 minutes. When 40 mg of tissue was stimulated by 10^{-6} M adrenaline, 75 picomoles cyclic AMP were recovered. The addition of phosphodiesterase to this endogenously stimulated cyclic AMP resulted in relatively complete destruction of this material.

TABLE
RECOVERY STUDIES OF CYCLIC AMP (c-AMP) IN
RAT LUNG TISSUE

	Picomoles c-AMP ¹ recovered in 15 min.
20 mg Tissue	19
100 mg Tissue	86
20 mg Tissue + 10 picomoles c-AMP	19 30
100 picomoles c-AMP + phosphodiesterase	100 5
40 mg Tissue + adrenaline 10^{-6} M	45 75
+ adrenaline + phosphodiesterase	10

¹Mean value, n = 4.

Figure 1 depicts a time study with adrenaline 10^{-5} M. Forty milligrams of rat lung yielded rapidly increasing amounts of cyclic AMP with a greater than 100% increase after 15 seconds' incubation. Peak stimulation to 175 picomoles cyclic AMP occurred at 2 minutes. Subsequent measurements at 5, 10, and 15 minutes showed a gradual decrease in recoverable cyclic AMP.

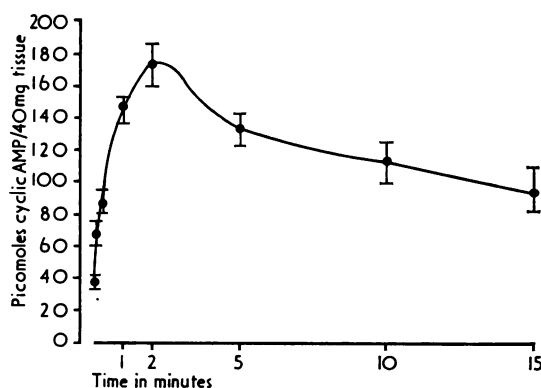


FIG. 1. Time study with adrenaline 10^{-5} M. Mean values with range (n=4). All values are significant from control at $P < 0.01$.

Figure 2 shows dose response curves for isoprenaline, adrenaline, noradrenaline, and ephedrine stimulation of cyclic AMP in rat lung. The addition of adrenaline at 10^{-4} M concentration produced an increase in cyclic AMP from 35 to 131 picomoles in 40 mg of tissue. Isoprenaline, in equivalent concentrations, demonstrated a greater stimulatory effect than adrenaline; noradrenaline was less stimulatory, and ephedrine was ineffective. The mean percentage increases above control at 10^{-5} M were approximately: isoprenaline 225%, adrenaline 200%, noradrenaline 80%, and ephedrine 5%.

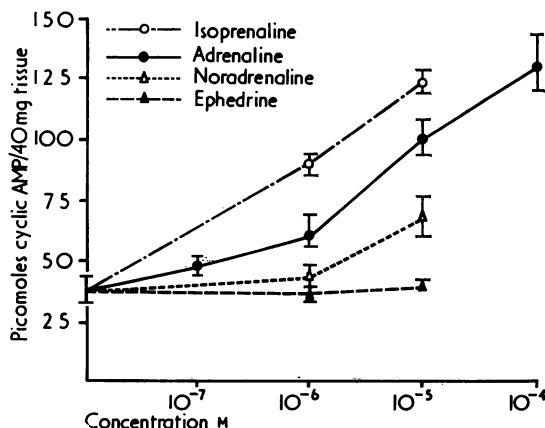


FIG. 2. Dose response curve with isoprenaline, adrenaline, noradrenaline, and ephedrine. All incubations were conducted for 15 minutes and tissue was handled as shown in methods. Mean values with range (n=4).

Figure 3 demonstrates the effects of phosphodiesterase inhibitors on cyclic AMP formation in rat lung. Caffeine 10^{-3} M resulted in approximately 100% increase in cyclic AMP. The addition of adrenaline 10^{-6} M to this system produced a synergistic effect, a 200% increase above control ($P < 0.05$). Similarly, theophylline 10^{-3} M increased cyclic AMP concentration but to a lesser extent than caffeine. The addition of adrenaline to this system resulted in a further increase (110% above control).

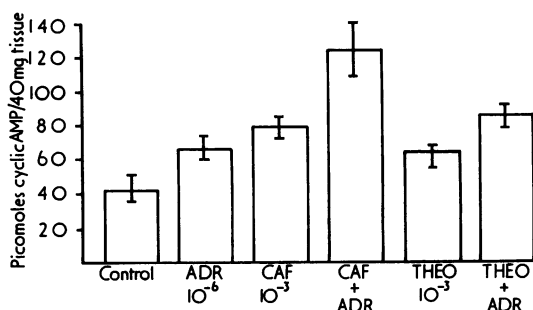


FIG. 3. Bar graph demonstrating effects of caffeine (Caf) 10^{-3} M and theophylline (Theo) 10^{-3} M alone and in combination with adrenaline (Adr) 10^{-6} M. All incubations were conducted for 15 minutes and tissue was handled as shown in methods. Mean values with range ($n=4$). Adr stimulates ($P < 0.05$), Caf alone stimulated ($P < 0.05$), and Caf + Adr stimulated ($P < 0.01$).

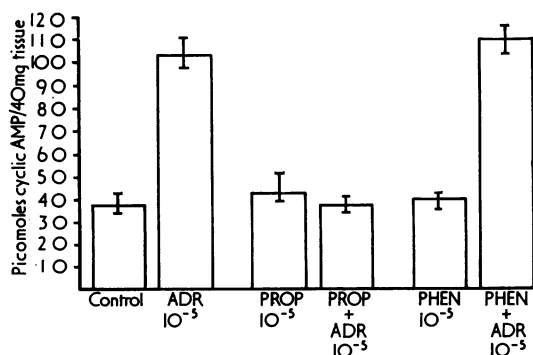


FIG. 4. Bar graph demonstrating effects of propranolol (Prop) 10^{-5} M and phentolamine (Phen) 10^{-5} M alone and in combination with adrenaline (Adr) 10^{-5} M. All incubations were conducted for 15 minutes and tissue was handled as shown in methods. Mean values with range ($n=4$). Adr stimulates ($P < 0.01$), Prop inhibits Adr ($P < 0.01$), and Phen has no effect (PNS).

Figure 4 illustrates the effects of alpha and beta adrenergic blocking agents on adrenaline stimulated rat lung. Propranolol, a beta blocker, at 10^{-5} M completely inhibited any increase in cyclic AMP in the adrenaline stimulated lung. In contrast, the alpha adrenergic blocking agent, phentolamine, did not inhibit the increase in cyclic AMP seen with adrenaline stimulation.

DISCUSSION

According to the second messenger hypothesis of hormonal action, as applied to lung, the major endogenous bronchodilator, adrenaline reacts with a receptor in the cell membrane activating adenylyl cyclase, and promoting conversion of ATP to adenosine 3', 5' cyclic monophosphate (cyclic AMP). Accumulation of this substance intracellularly produces many effects in different cells, i.e., in catecholamine-sensitive tissue such as lung, an increase in cyclic AMP inhibits the release of mediators of bronchospasm, such as histamine and slow-reacting substance of anaphylaxis (SRS-A (Middleton, 1972)).

Cyclic AMP is broken down to 5'-AMP by the enzyme cyclic nucleotide phosphodiesterase. Therefore, intracellular concentrations of cyclic AMP are determined by the balance between production via adenylyl cyclase and destruction via phosphodiesterase. Because the pieces of rat lung used in these experiments contain many diverse cells, one cannot determine the relative contribution of the various cell types to the total amount of cyclic AMP produced. Nevertheless it is useful to know how 'whole lung tissue' reacts *in vitro*. The present experiments demonstrate that cyclic AMP can be recovered from pieces of rat lung in a concentration approximating 1 picomole cyclic AMP per milligram wet weight of lung tissue. Although the methods employed in these studies were somewhat different from those used by Sutherland *et al.* (1968), the amount of cyclic AMP recovered from the two systems was quite similar. As demonstrated in the Table, exogenously added cyclic AMP was recovered in a weight-related fashion. Furthermore, incubation with phosphodiesterase resulted in almost complete destruction of both exogenous and endogenously stimulated immunoreactive cyclic AMP. This confirms the specificity of the assay.

The time study demonstrates an instantaneous stimulatory effect of adrenaline with a 340% increase in cyclic AMP concentration at 2 minutes. The dose response experiments showed isoprenaline, in equivalent concentration, to

produce a greater stimulation of cyclic AMP than adrenaline which, in turn, was more potent than noradrenaline. These findings agree with the previously reported work of Kaliner *et al.* (1971). Our data are the first reporting the effect of ephedrine, probably the most commonly used drug in the treatment of bronchospastic disorders. In this model system ephedrine, even at high concentration, showed little stimulatory effect on cyclic AMP production.

The phosphodiesterase inhibitors, caffeine and theophylline, increased cyclic AMP concentration in lung tissue. Caffeine was more potent than theophylline in an equivalent concentration, producing respectively 100% versus 50% increases in cyclic AMP. Augmented cyclic AMP formation was observed with adrenaline in combination with either caffeine or theophylline. These data fulfil many of the criteria of Sutherland's second messenger hypothesis in that cyclic AMP concentration in intact tissue increases after hormonal stimulation, and that hormones which stimulate adenyl cyclase are potentiated by drugs which inhibit phosphodiesterase activity (Sutherland, 1972).

In agreement with previous investigators (Kaliner *et al.*, 1971; Orange *et al.*, 1971; Palmer, 1971) it has been shown that the increased levels of the lung cyclic AMP induced by the beta adrenergic stimulant, adrenaline, were antagonized by the beta adrenergic blocker, propranolol. In addition, we demonstrated that the alpha adrenergic blocking agent, phentolamine, had no effect on adrenaline-induced cyclic AMP response (Palmer, 1971). Neither propranolol nor phentolamine alone affected basal levels of cyclic AMP.

In catecholamine-sensitive tissues the principal bronchodilator drugs, sympathomimetic amines and methylxanthines, act via pathways that tend to increase intracellular concentrations of cyclic AMP. The prevalent theory of the pathophysiology of bronchospastic disorders, that of 'beta-adrenergic blockade', holds that a diminished catecholamine responsiveness exists in the beta-adrenergic receptors of the cell membrane. Since this receptor is apparently closely associated with adenyl cyclase, 'blockade' could be associated with a reduction in cyclase activity and decreased production of cyclic AMP (Middleton, 1972). Clinical investigation supports a less than expected response in asthmatic subjects to the administration of sympathomimetic amines, with physiological alterations mirroring those of normal subjects treated with the beta blocker propranolol.

Adrenaline and theophylline also inhibit the antigen-induced release of histamine and SRS-A in vitro from sensitized human lung (Orange *et al.*, 1971). Pharmacological manipulations favourable to intracellular accumulation of cyclic AMP consistently decrease the release of these mediators of bronchospasm, whereas compounds that reduce cyclic AMP concentrations in the lung increase the release of these mediators (Middleton, 1972).

These experiments may provide new insight into the effectiveness of old antiasthmatic drugs previously described as combating mucosal oedema, smooth muscle contraction, and mucous gland hypersecretion in bronchospastic disorders. It would also appear from the literature that inhibition of mediator release is essential to the activity of these drugs, and that this mechanism of inducing physiological change is modulated by metabolic alterations of intracellular cyclic AMP.

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