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CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE RESPONSE IN THE RABBIT LUNG— ADULT PROPERTIES AND DEVELOPMENT

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Abstract—When various doses of catecholamines (norepinephrine, epinephrine and isoproterenol) and histamine were added to incubated rabbit lung slices, there was a dose-related increase in levels of cyclic 3′,5′-adenosine monophosphate (cAMP). Isoproterenol was the most potent and histamine the least potent agent capable of eliciting this response. The time course of this hormonally induced cAMP response was maximal from 6–36 min. Propranolol but not phentolamine significantly antagonized the catecholamine-induced cAMP response, while tripelennamine blocked the effects of histamine. Aminophylline enhanced the catecholamine and histamine stimulation of cAMP. During lung development, a cAMP response to epinephrine was detected at fetal day 21. By fetal day 25 a prominent cAMP response to epinephrine, norepinephrine and histamine occurred. The response to catecholamines dropped slightly at birth, and the highest cAMP levels were seen at 4 days postpartum. The histamine-induced response was maximal at fetal day 28, was diminished at birth and another peak response was observed at 20 days postpartum.

IT HAS been established that cyclic 3′,5′-adenosine monophosphate (cAMP) mediates specific hormone responses in a variety of tissues.[1] At present there are few studies concerning the role of the cyclic nucleotide in lung tissue. The lung has been shown to have an active adenylate cyclase–phosphodiesterase–protein kinase enzyme system.[2–4] Some recent studies have indicated that a variety of hormones, namely the catecholamines, histamine and prostaglandin E_1 stimulate the production of cAMP in lung tissue from rat, guinea pig and humans.[5–7] In preliminary studies it was shown that chlorpromazine antagonized the cAMP response elicited by epinephrine and histamine in the rat and guinea pig lung.[5] The effects of catecholamines were blocked by propranolol in the human, rat and guinea pig lungs, while phentolamine antagonized the epinephrine-induced cAMP response in the guinea pig. The antihistamine, tripelennamine, blocked the cAMP response in the rat lung.[5,6]

The aim of the present study was to investigate in greater detail the pharmacological properties of the cAMP response in the adult rabbit lung, bearing in mind the possibility that a species difference might exist concerning the effect of specific hormones. Since no data are available on the pattern of development of the stimulation of cAMP to hormones in the lung, this parameter was also investigated.

MATERIALS AND METHODS

The essential details of the experimental methods have been described.[5,8,9] Pregnant white New Zealand rabbits were sacrificed by cervical dislocation, and the lungs and

hearts from the mothers and fetuses were rapidly removed and placed in cold Krebs–Ringer bicarbonate buffer. The lungs were sliced into approximately 2-mm cubes rinsed three times in buffer, placed in a Dubnoff metabolic shaker and preincubated at 37° while O_2 containing 5% CO_2 was bubbled continuously into individual samples. Thirty min later the buffer was changed (20 ml added), and at this time whenever applicable either blocking agents, aminophylline or a control solution were added to the samples. After an additional 15-min incubation, the designated hormone was introduced to the samples. At specific time intervals the contents of the incubation flask were poured into an operating Waring blender containing 2 ml of 1 N HCl. An aliquot was removed for protein (Biuret method) and the remainder of the homogenate was centrifuged at 8000 *g* for 20 min. The isolation and analysis of cAMP were carried out by the procedure described by Butcher *et al.*[8] All samples were assayed in triplicate at two dilutions, and cAMP was expressed as picomoles per milligram of sample protein. Basal as well as hormonally activated cAMP levels tended to vary with the different enzyme preparations utilized in the assay. In order to obtain consistent results, a group of 4–8 animals was selected for individual experiments depicted in each figure or table using the same enzyme preparation. Thus each figure or table contained its own set of controls, hormone responses and where applicable aminophylline or antagonists. In the fetal studies, fetuses from two pregnant mothers were always used. Several samples were pooled at fetal day 21, two fetuses per sample utilized at fetal days 25 and 28 and thereafter samples from individual animals were used. The Student's *t*-test was utilized for statistics, and a P value of 0·05 was the upper limit of significance.

Chemicals. Histamine, *l*-epinephrine and *d,l*-norepinephrine were obtained in the hydrochloride form, dissolved in bovine serum albumin (40 μg/ml) and purchased from Sigma. Phentolamine HCl (Regitine), *d,l*-propranolol HCl and tripelennamine HCl (Pyribenzamine) were purchased from Sigma and dissolved in water; *d,l*-isoproterenol HCl was dissolved in bovine serum albumin (40 μg/ml) and purchased from Aldrich Chem. Co. Prostaglandins E_2, F_2d and A_2 were a gift from Dr. John E. Pike from The Upjohn Company. Aminophylline (theophylline-ethyienediamine) was purchased from Matheson Coleman & Bell and was dissolved in water.

RESULTS

Characteristics of the adult response

Dose–response. With incubated adult lung slices, Fig. 1 shows that the catechol. amines were capable of stimulating cAMP to a 15-fold maximum within 6 min. Isoproterenol-induced cAMP production was maximal from 5×10^{-7} to 5×10^{-4} M while a lesser but significant ($P = < 0{\cdot}05$) elevation occurred at 5×10^{-8} M. Epinephrine from 5×10^{-5} to 5×10^{-4} M elicited the maximum cAMP response with significant increases observed at doses of 5×10^{-7} to 5×10^{-6} M. The maximal cAMP response to norepinephrine was 5×10^{-4} M; however, doses from 5×10^{-7} to 5×10^{-5} M significantly raised the levels of the cyclic nucleotide. From the data shown in Fig. 1, it appears that doses of norepinephrine and epinephrine at 5×10^{-8} M inhibit the formation of cAMP, but these results do not differ significantly ($P = > 0{\cdot}05$) from the control values.

The maximal stimulation of cAMP in the presence of histamine (Fig. 2, Panel A) occurred from 5×10^{-5} to 5×10^{-4} M concentration, but the overall magnitude was

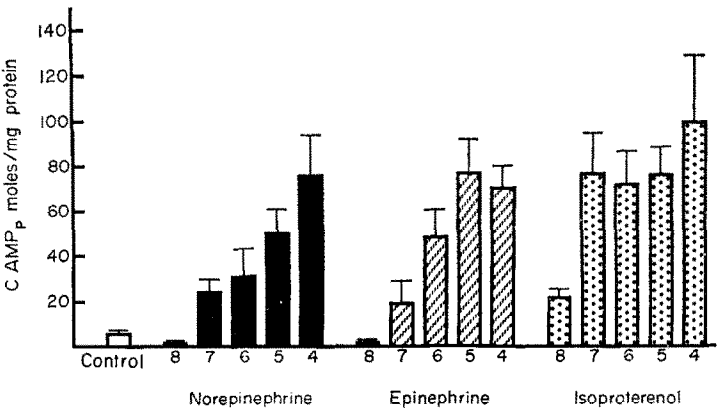

FIG. 1. Dose–response relationships of the catecholamine-induced cAMP response in the adult rabbit lung *in vitro*. Lung slices were incubated with various doses of catecholamines for 6 min. cAMP levels were determined and expressed as picomolar per milligram of sample protein. The bars represent the mean of 4–5 separate animals and the vertical lines are S.E.M. The numbers represent molar doses; 8 = 5×10^{-8}, 7 = 5×10^{-7}, 6 = 5×10^{-6}, 5 = 5×10^{-5} and 4 = 5×10^{-4}

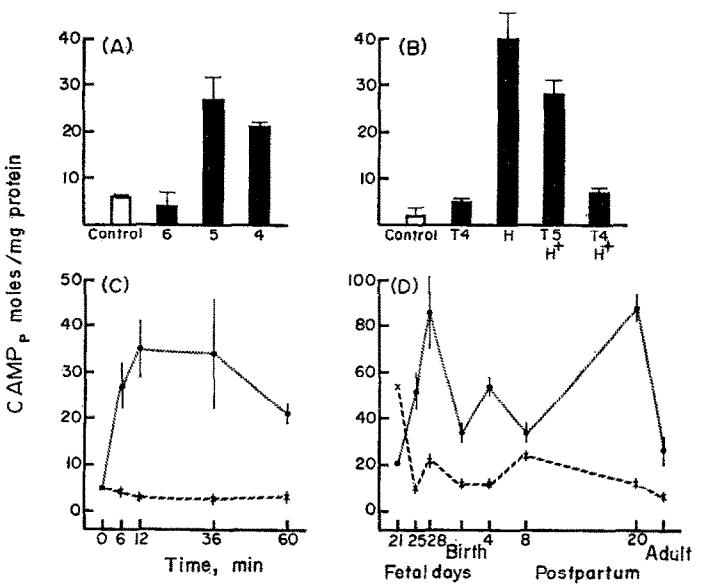

FIG. 2. Characteristics of the histamine-induced cAMP response in the adult and developing rabbit lung *in vitro*. Lung slices were incubated in the presence of histamine, blocking agents or control solutions according to the following conditions. Cyclic AMP levels are expressed as picomoles per milligram of sample protein. The values represent the mean of 4–5 separate animals and the vertical lines are S.E.M. Panel (A) Dose–response relationship after adding various amounts of histamine followed by 6-min incubation. The numbers represent doses; 6 = 5×10^{-6} M, 5 = 5×10^{-5} M and 4 = 5×10^{-4} M. Panel (B) Effect of tripelennamine on the stimulation of cAMP by histamine (5×10^{-5} M for 6 min). Abbreviations are: T = tripelennamine, H = histamine, 5 = tripelennamine at 5×10^{-5} M and 4 = tripelennamine at 5×10^{-4} M. Panel (C) Time course of the histamine-induced stimulation of cAMP. Closed circles = histamine (5×10^{-5} M); X = control samples. Panel (D) Development of the histamine-induced stimulation of cAMP. Closed circles = histamine (5×10^{-5} M); X = control samples.

one third that observed for the catecholamines. From the dose–response studies, the order of potency for cAMP stimulation in the adult lung was: isoproterenol > epinephrine > norepinephrine > histamine.

Time course. As shown in Fig. 3, the time courses for the accumulation of cAMP by the lung slices in the presence of individual catecholamines (norepinephrine, epinephrine and isoproterenol at 5×10^{-5} M) were nearly identical with no significant changes occurring among these agents at any period. Maximal levels of cAMP were produced from 6 to 36 min of incubation with a drop noted by 60 min. During this time, basal levels of the cyclic nucleotide were not altered. The pattern of the cAMP response to histamine (Fig. 2, Panel C) was somewhat similar, but overall cAMP levels did not reach the magnitude of that observed with the catecholamines.

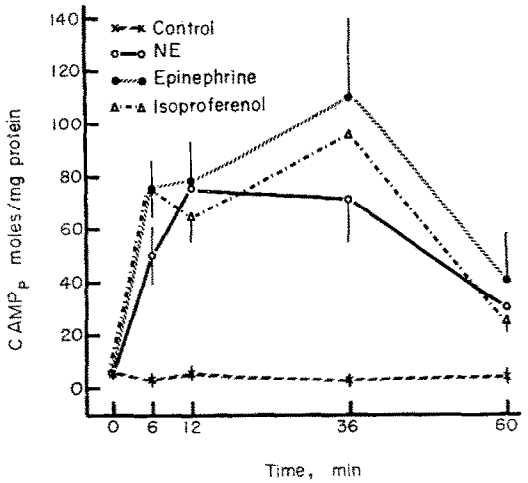

FIG. 3. Time course of the catecholamine-induced cAMP response in the adult rabbit lung *in vitro*. Lung slices were incubated for various times in the presence of catecholamines (5×10^{-5} M) and cAMP is determined and expressed as picomoles per milligram of sample protein. The values represent the mean of 4–5 separate animals and the vertical lines are S.E.M.

Effect of aminophylline. As seen in Table 1, the presence of aminophylline (2×10^{-3} M) significantly enhanced (3- to 4-fold) the hormonally induced accumulation of cAMP. Basal levels of the cyclic nucleotide were also elevated in the presence of aminophylline.

Effect of blocking agents. As shown in Fig. 4, propranolol (5×10^{-5} M) significantly antagonized the cAMP response elicited by equal molar doses of catecholamines. Phentolamine (5×10^{-5} M), an alpha blocking agent, also antagonized the 6-min stimulation of cAMP by epinephrine and norepinephrine (5×10^{-5} M), but these results were not significant. No antagonism of the isoproterenol-induced response by phentolamine was observed. Neither adrenergic blocking agent altered basal levels of cAMP (Fig. 4). The antihistamine tripelennamine, at 5×10^{-4} M significantly blocked the cAMP response to histamine (5×10^{-5} M, 6 min) without affecting basal levels of the cyclic nucleotide (Fig. 2, Panel B).

Effect of prostaglandins. Large doses of the prostaglandins E_2, A_2 and F_2d (5×10^{-5} M) did not affect the levels of cAMP in the rabbit lung following 6-min incubation. Values (picomoles of cAMP per milligram of protein) are the mean of five

TABLE 1. EFFECT OF AMINOPHYLLINE ON THE HORMONAL-INDUCED cAMP RESPONSE IN THE ADULT RABBIT LUNG *in vitro**

Treatment	cAMP (pmoles/mg sample protein)	
	Effect of hormones alone	Effect of aminohylline + hormones
Control	3·8 ± 1·6	6·4 ± 1·9 NS
Epinephrine	52·3 ± 8·2	181·8 ± 15·0 (P = <0·0001)
Isoproterenol	34·1 ± 4·8	107·9 ± 37·0 (P = <0·05)
Norepinephrine	36·3 ± 6·6	132·3 ± 21·9 (P = <0·05)
Histamine	24·9 ± 3·4	118·4 ± 26·6 (P = <0·01)

* Lung slices were preincubated in the presence of either aminophylline (2×10^{-3} M) or a control solution. Either hormones (5×10^{-5} M) or a control solution were then added and 6 min later the incubations were terminated and cAMP levels determined. The values are means of four separate animals ±S.E.M. P values represent samples incubated with aminophylline plus hormones compared to that specific hormone alone. NS = not significant.

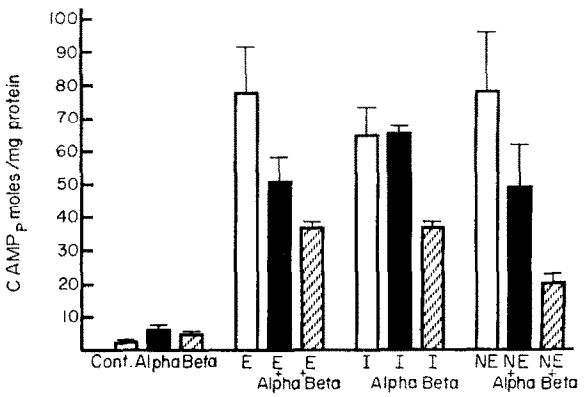

FIG. 4. Effect of adrenergic blocking agents on the catecholamine-induced cAMP response in the adult rabbit lung *in vitro*. Lung slices were incubated 15 min with either propranolol (beta) or phentolamine (alpha) at 5×10^{-5} M or a control solution. The catecholamines (E = epinephrine, I = isoproterenol, and NE = norepinephrine) at 5×10^{-5} M or a control solution were then added and 6 min later the incubations were terminated and cAMP levels were determined and expressed as picomoles per milligram of sample protein. The bars represent the mean of 4–5 separate animals and the vertical lines are S.E.M. The antagonism by propranolol (beta) of the catecholamine-induced response was significant (Student's *t*-test) and P values are: E, P = <0·05; I, P = <0·05; and NE, P = <0·01.

experiments ±S.E.M. Control = 5 ± 1, E_2 = 6 ± 1·6, A_2 = 7·5 ± 2 and $F_2\alpha$ = 6 ± 1.

Developmental studies. The results of the development of the cAMP response to hormones are depicted in Figs. 5 and 2 (Panel D). All incubations were for 6 min with 5×10^{-5} M hormones. By fetal day 21, a large cAMP response was observed to epinephrine in a pooled sample from several fetuses; however, an inhibitory response

to histamine appeared to occur at this time. From fetal day 25 to birth, epinephrine and norepinephrine stimulated cAMP to almost adult levels. The greatest cAMP response to catecholamines was seen at 4 days postpartum which dropped to adult levels by 8 days. The histamine-induced cAMP response followed a more irregular pattern. A large response was observed at fetal day 28 which dropped at birth, and increased slightly thereafter with another peak observed at 20 days postpartum. Basal levels of the cyclic nucleotide were highest at fetal day 21 but did not change to any degree during subsequent development of the lung.

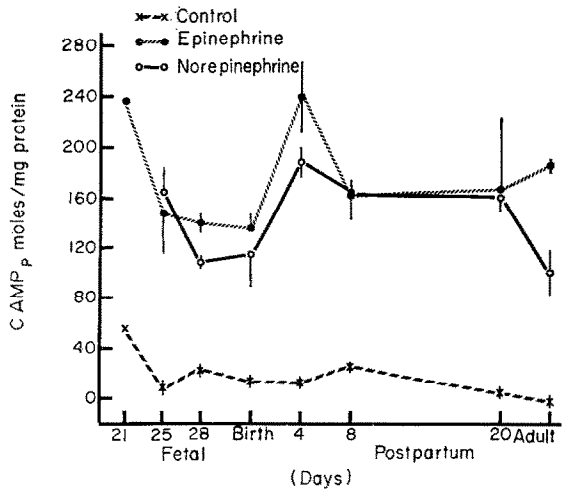

FIG. 5. Development of the epinephrine and norepinephrine-induced cAMP response in the rabbit lung. Fetal lung slices were incubated in the presence of either epinephrine or norepinephrine (5×10^{-5} M) for 6 min and cAMP levels were determined and expressed as picomoles per milligram of sample protein. The values represent the mean of 3–4 separate animals and the vertical lines are S.E.M.

Heart studies. The cAMP response in heart slices from fetal day 28 to the adult was measured in the presence of either norepinephrine (5×10^{-5} M) or glucagon (1 μg/ml). There was no cAMP response to either agent at any time period except in the adult when norepinephrine elicited a 3-fold increase (control = 3·3 pmoles/mg protein and norepinephrine = 9·5 pmoles/mg protein, mean of two experiments). The enzyme adenylate cyclase must have been present during these stages of development because basal levels of cAMP were detected at all time periods.

DISCUSSION

In the present experiments the bronchial dilating agents, isoproterenol, epinephrine and norepinephrine were more potent than histamine in their ability to stimulate the accumulation of cAMP in the rabbit lung *in vitro*. Dose–response relationships revealed that isoproterenol was the most potent and that norepinephrine was the least potent catecholamine in this regard. The effects of the three catecholamines on cAMP production were significantly antagonized by equal molar amounts of the beta adrenergic blocking agent, propranolol. The alpha blocking agent, phentolamine, was without significant effect in these experiments. Aminophylline, an inhibitor of cAMP-phosphodiesterase, enhanced the cAMP response to the catecholamines. These

results confirm to some extent preliminary studies by this author[5] and others.[6,7] It was found in human lung *in vitro* that isoproterenol was the most powerful and norepinephrine the least powerful catecholamine that stimulated the conversion of ^{14}C adenine to labeled cAMP. The effects of the catecholamines were antagonized by propranolol and enhanced by aminophylline.[6] It was further shown using spirally cut bronchial strips from dogs and guinea pigs that isoproterenol, epinephrine and norepinephrine in this order of potency relaxed bronchial smooth muscle as well as induced cAMP formation. Propranolol blocked the effects of these catecholamines.[10] In a recent report, it was shown that propranolol and chlorpromazine antagonized the cAMP response to epinephrine in the rat and guinea pig lung *in vitro*.[5] Taken together these results support the hypothesis that beta adrenergic receptors mediate increased levels of cAMP while the function of the alpha receptor has not been clarified.[1]

In the time course studies, in the presence of either catecholamines or histamine cAMP levels accumulated up to 36-min incubation, in spite of the absence of a phosphodiesterase inhibitor. Similar observations have been observed using slices of rabbit and guinea pig brain.[11] On the other hand, Exton *et al.*[12] and Robison *et al.*[13] using perfused liver and heart preparations found that the maximal cAMP production in response to glucagon and catecholamines occurred within 2 min and declined rapidly thereafter. The basis for this discrepancy is at present unknown.

Histamine which has been shown to be a powerful contracting agent on bronchial smooth muscle also elevated cAMP levels in the rabbit lung *in vitro*. This response was less than that seen using the catecholamines, but nevertheless significant. The histamine response was enhanced by aminophylline and antagonized by the antihistamine, tripelennamine. A somewhat similar observation was reported in the rat and guinea pig lungs, except that histamine was as potent as epinephrine. This may suggest a species difference in the ability of the lung to respond metabolically to histamine. In these studies chlorpromazine and tripelennamine blocked the cAMP response to histamine in only the rat lung.[5]

The differential pattern of development of the cAMP response to the two classes of hormones may be attributed to either maturation of the adenyl cyclase receptor in the diverse cellular populations found in the lung or separate receptors. A somewhat similar observation was seen during development of the activation of adenylate cyclase by hormones in the rat pineal.[14] In the present study, an enhanced cAMP response to catecholamines occurred 4 days after birth and to histamine at neonatal day 20. Thereafter the activation of cAMP declined to the adult level. Analogous observations have been reported during the development of the cAMP response to norepinephrine and histamine in the rat and rabbit brain.[15–17] Phosphodiesterase was also shown to be active at this time of development.[15] These authors postulated that the immature brain cells possess a hyperactive adenylate cyclase receptor lacking in either a neuronal or intracellular control element that was responsible for the increased cAMP response.[16]

In earlier studies utilizing perfused hearts, there was observed a rapid increase (6-fold) in cAMP levels in response to catecholamines[13] and glucagon.[18] In the present study using adult cardiac slices, there was only a slight increase in cAMP in response to norepinephrine but not to glucagon. An almost identical study by Laraia and Reddy[19] also showed that only catecholamines in the presence of caffeine were capable of eliciting a 2-fold elevation of cAMP. It seems that during preparation the cardiac

slice loses its sensitivity to hormones. However, a recent report utilizing a new technique for slice incubation demonstrated a cAMP response to both catecholamines and glucagon to the magnitudes observed in the perfusion studies.[20] These results may explain the failure reported in the present experiments.

The present studies demonstrate that an active cAMP response to selective hormones is present in the adult lung and that these responses appear at different stages during development. However, the role of cAMP on subsequent metabolic events in the developing or adult lung is unknown. A possible role during development might be induction of fetal enzymes as suggested by the studies using fetal liver and fat cells.[21–23] In addition, the fetal lung contains abundant stores of glycogen,[24] and a role for cAMP in fetal carbohydrate metabolism could be expected. Further experiments are needed to elucidate these complicated mechanisms.

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