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**Research Article**

Human umbilical arteries are unique vessels in that they close quickly and completely at birth. It has been suggested that cyclic uanosine 3',5'-monophosphate (cAMP) in relaxation. This hypothesis has been evaluated in term gestational human umbilical artery segments incubated at 37 degrees C and in room air. (a) The basal cGMP content (1 pmol/mg protein) of artery segments incubated in room air was almost twice that of cAMP. (b) Bradykinin, histamine, serotonin, acetylcholine, and K<sup>+</sup> ion, which cause umbilical artery constriction, can increase the cGMP content of the artery segments within 30 s of exposure without altering the cAMP content. (c) Prostaglandin E1, but not isoproterenol, caused accumulation of cAMP which is consistent with reports that umbilical arteries lack functional beta-receptors and that only prostaglandin E1 can bring about relaxation of umbilical arteries. (d) 1 muM atropine blocked the effect of 100 muM acetylcholine on cGMP content without altering the responses to histamine, bradykinin, serotonin, or K<sup>+</sup> ion. (e) Ppyrilamine (an H1 antagonist), but not metiamide (an H2 antagonist), blocked the effect of histamine on cGMP from which it is inferred that histamine causes accumulation of cGMP in umbilical artery via its interaction with H1 receptors. The results are consistent with the view that metabolism of the two cyclic nucleotides is independently controlled in the human umbilical artery and that [...]

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# Guanosine 3', 5'-Monophosphate and Adenosine 3', 5'-Monophosphate Content of Human Umbilical Artery

## POSSIBLE ROLE IN PERINATAL ARTERIAL PATENCY AND CLOSURE

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**ABSTRACT** Human umbilical arteries are unique vessels in that they close quickly and completely at birth. It has been suggested that cyclic guanosine 3',5'-monophosphate (cGMP) plays a role in smooth muscle contraction and cyclic adenosine 3',5'-monophosphate (cAMP) in relaxation. This hypothesis has been evaluated in term gestational human umbilical artery segments incubated at 37°C and in room air. (a) The basal cGMP content (1 pmol/mg protein) of artery segments incubated in room air was almost twice that of cAMP. (b) Bradykinin, histamine, serotonin, acetylcholine, and K<sup>+</sup> ion, which cause umbilical artery constriction, can increase the cGMP content of the artery segments within 30 s of exposure without altering the cAMP content. (c) Prostaglandin E<sub>1</sub>, but not isoproterenol, caused accumulation of cAMP which is consistent with reports that umbilical arteries lack functional β-receptors and that only prostaglandin E<sub>1</sub> can bring about relaxation of umbilical arteries. (d) 1 μM atropine blocked the effect of 100 μM acetylcholine on cGMP content without altering the responses to histamine, bradykinin, serotonin, or K<sup>+</sup> ion. (e) Pyrilamine (an H<sub>1</sub> antagonist), but not metiamide (an H<sub>2</sub> antagonist), blocked the effect of histamine on cGMP from which it is inferred that histamine causes accumulation of cGMP in umbilical artery via its interaction with H<sub>1</sub> receptors. The results are consistent with the view that metabolism of the two cyclic nucleotides is independently controlled in the human umbilical artery and that cGMP is involved in contraction of the artery at birth.

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## INTRODUCTION

Umbilical arteries are unique blood vessels in the sense that they must remain patent *in utero* but most close completely and relatively quickly after delivery. The precise mechanism for the spontaneous closure of the umbilical vessels at birth is unknown. The importance of neurogenic mechanisms may be questionable, since it is thought that umbilical arteries are either not innervated (1) or are associated with only a scanty supply of nerve elements (2).

It is generally believed that humoral agents or oxygen or both are the stimuli that initiate closure of umbilical arteries. The artery *in vitro* constricts when exposed to oxygen (3-6). In addition, by virtue of their ability to produce constriction *in vitro* as well as their actual or presumed concentrations in cord blood at birth, bradykinin, serotonin, histamine, acetylcholine, prostaglandins A<sub>2</sub> and F<sub>2α</sub> have been suggested as probable chemical mediators of umbilical artery closure (4-10). Several of these agents have been reported to cause accumulation of cyclic guanosine 3',5'-monophosphate (cGMP)<sup>1</sup> in other tissues (11-16). We have found, as reported below, that bradykinin, histamine, serotonin, and acetylcholine, as well as potassium chloride, in concentrations that cause contraction (4, 6), can increase the cGMP content of the arteries without altering the cAMP content. Accumulation of cAMP was induced by prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) but not by isoproterenol.

<sup>1</sup> *Abbreviations used in this paper:* cAMP, adenosine 3',5'-monophosphate; cGMP, guanosine 3',5'-monophosphate; PGE<sub>1</sub>, PGA<sub>1</sub>, PGA<sub>2</sub>, PGF<sub>2α</sub>, prostaglandins E<sub>1</sub>, A<sub>1</sub>, A<sub>2</sub>, and F<sub>2α</sub>.

These observations are consistent with earlier reports that umbilical arteries lack functional  $\beta$ -adrenergic receptors (4, 7) and that PGE<sub>1</sub>, but not isoproterenol, can bring about dilatation or relaxation of umbilical arteries (9).

## METHODS

Human umbilical cords from normal full-term deliveries were obtained within 30 min of delivery. Both arteries were dissected and prepared for incubation within 2 h. Hanks' medium at room temperature was used for these procedures. As preliminary studies revealed no difference in effects of various agonists on the cGMP content of arterial segments taken from placental, midcord, and infant thirds of the same artery, the entire length of the artery, except for 5 cm at either end, was used. The arteries were opened longitudinally, divided into 0.5-cm segments which were randomly assigned to 25-ml Erlenmeyer flasks containing 4 ml of a modified Krebs-Ringer-Tris solution (127 mM NaCl, 5 mM KCl, 2.7 mM CaCl<sub>2</sub>, 1.27 mM KH<sub>2</sub>PO<sub>4</sub>, 1.27 mM MgSO<sub>4</sub>, glucose, 1 mg/ml, 15 mM Tris buffer, pH 7.3, with albumin [fraction V from bovine serum], 100  $\mu$ g/ml). Approximately 300–400 mg of tissue was placed in each flask and incubated for 2–3 h in room air at 37°C. Additions in 100- $\mu$ l volumes were then made, and at the indicated time thereafter incubation was terminated by addition of 1 ml of cold 12% perchloric acid followed by homogenization of tissue and medium with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) and centrifugation at 3,200 *g* for 30 min. The precipitates dissolved in 1 N NaOH were used for determination of protein (17) with bovine serum albumin as a standard. Protein content was 4.5–5.5% of wet tissue weight.

After the addition of [<sup>3</sup>H]cGMP (<1 pmol) to each, supernates were neutralized with 50% KOH and centrifuged. The supernates were applied to columns (0.5 × 3 cm) of neutral alumina which had previously been washed with 10 ml 5 mM Tris-HCl buffer, pH 7.4, and followed by 6 ml of the same buffer. The resulting eluates were applied to columns (0.5 × 3 cm) of AG 1-X8 formate which were then washed with 20 ml of 0.5 N formic acid before elution of cGMP with 10 ml of 4 N formic acid. The eluates were lyophilized, and the residues dissolved in 500  $\mu$ l of 50 mM sodium acetate buffer, pH 6.2, with 1.6 mM MgCl<sub>2</sub>. Samples were taken for determination of recovery of [<sup>3</sup>H]cGMP which ranged from 45 to 65% and for radioimmunoassay of cGMP (18).

For each concentration point on the standard curve, we carried a sample of incubation medium plus perchloric acid through the purification process used for the tissue extracts. These lyophilized residues were dissolved in 500  $\mu$ l buffer, and an appropriate standard of cGMP was added to each. Samples of unknowns and of these cGMP standards were incubated with labeled antigen and antibody in 50 mM sodium acetate, pH 6.2, with 1.6 mM MgCl<sub>2</sub> at 4°C for 16–24 h. 500  $\mu$ g Rabbit serum  $\gamma$ -globulin (19) was then added to each sample and the bound antigen precipitated with ammonium sulfate (final concentration equal to 30 g/100 ml). After centrifugation at 1,500 *g* for 45 min, the supernates were decanted. The precipitates were washed again with ammonium sulfate solution before assay of <sup>125</sup>I in a Packard Auto Gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). This procedure yielded reproducible standard curves with linear titration from 0.2 to 20 pmol of cGMP. Incubation of samples from representative experi-

ments with cyclic 3',5'-nucleotide phosphodiesterase before assay produced 100% hydrolysis of cGMP in all cases.

For cAMP experiments, incubations were carried out as described and cAMP was purified as previously described (20) for assay by the method of Gilman (21).

[<sup>3</sup>H]cGMP (4.45 Ci/mmol) and [<sup>3</sup>H]cAMP (16.3 Ci/mmol) were purchased from Schwarz Bio Research Inc. (Orangeburg, N. Y.) and purified by thin-layer chromatography on cellulose with ethanol:0.5 M NH<sub>4</sub> acetate, 5:2 (vol/vol). cGMP, cAMP, cyclic 3',5'-nucleotide phosphodiesterase, neutral alumina (WN-3), acetylcholine bromide, histamine dihydrochloride, serotonin creatinine sulfate, L-isoproterenol bitartrate, and atropine sulfate were purchased from Sigma Chemical Co. (St. Louis, Mo.); theophylline from Nutritional Biochemicals Corp. (Cleveland, Ohio); fraction V from bovine serum from Armour Pharmaceutical Company (Chicago, Ill.); AG 1-X8 formate (200–400 mesh) from Bio-Rad Laboratories (Richmond, Calif.); bradykinin from Schwarz/Mann Div. (Becton, Dickinson & Co., Orangeburg, N. Y.); and materials for the cGMP assay from Collaborative Research, Inc. (Waltham, Mass.). Pyrilamine maleate (K & K, Plainville, N. Y.) was kindly provided by Dr. Jerome Fleisch, metamide by Dr. M. Beaven, and prostaglandins E<sub>1</sub>, A<sub>2</sub>, and F<sub>2 $\alpha$</sub>  by Dr. J. E. Pike of The Upjohn Company (Kalamazoo, Mich.). Stock solutions of prostaglandins were prepared in ethanol. The final concentration of ethanol in the medium in the prostaglandin experiments was 0.5% which was without effect on the cyclic nucleotide content of the artery segments.

## RESULTS

The cGMP content of 30 umbilical artery preparations incubated for 2 h in basal medium was 1.00 ± 0.08 pmol/mg protein (mean ± SEM). As shown in Fig. 1, within 0.5 min after the addition of 100  $\mu$ M acetylcholine, 1  $\mu$ M histamine, or 1.1  $\mu$ M bradykinin to the artery segments, marked accumulation of cGMP had occurred. Maximal accumulation of cGMP was attained within 2 min of exposure to the agonist. The effect of histamine was apparently sustained for at least 10 min, but those of acetylcholine and bradykinin decreased after 2 min. In further studies with these and other agents, a 2-min period of exposure was used.

In the experiments shown in Fig. 1, it is obvious that the effects of the three agonists differed greatly in magnitude, and there were also large differences in the effects produced by any one of them on different arteries (Fig. 2). The mean increments in cGMP produced by 1.1  $\mu$ M bradykinin and 1  $\mu$ M histamine were, however, greater than those of acetylcholine or serotonin (Fig. 2). In addition, when effects of two or more of the agonists were compared in any single experiment, those of 1.1  $\mu$ M bradykinin and 1  $\mu$ M histamine tended to be similar and consistently greater than those of 100  $\mu$ M acetylcholine or 10  $\mu$ M serotonin. In the experiment shown in Fig. 3, the effects of 1  $\mu$ M histamine and of 1.1  $\mu$ M bradykinin on arterial cGMP were less than maximal, although in other experiments these concentrations did produce apparently maximal accumulation

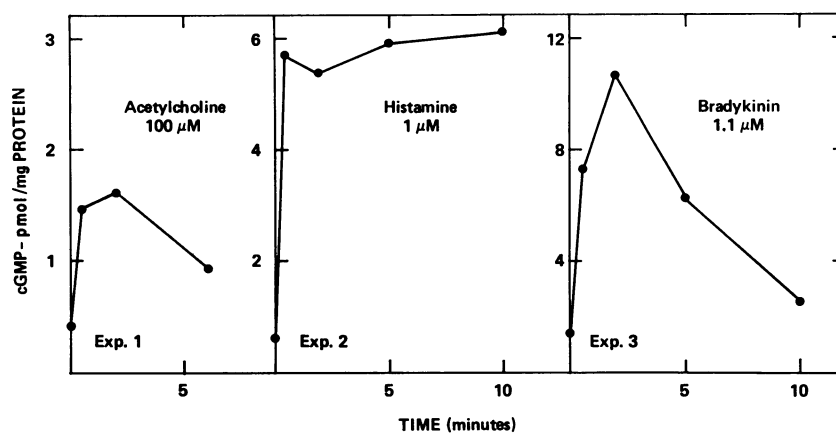


FIGURE 1 Effects of acetylcholine, histamine, and bradykinin on cGMP content of artery segments. In each experiment segments of an umbilical artery from a single cord were incubated for 2 h before addition of acetylcholine in exp. 1, histamine in exp. 2, and bradykinin in exp. 3. Incubations were terminated at the indicated times. Each point represents the mean cGMP content of duplicate samples of tissue plus medium.

of cGMP. In all experiments, essentially maximal effects of acetylcholine were produced with a concentration of 100  $\mu\text{M}$  whereas 10  $\mu\text{M}$  produced only 58% of the maximal effect. Serotonin, at a concentration of 10  $\mu\text{M}$ , was not maximally effective.

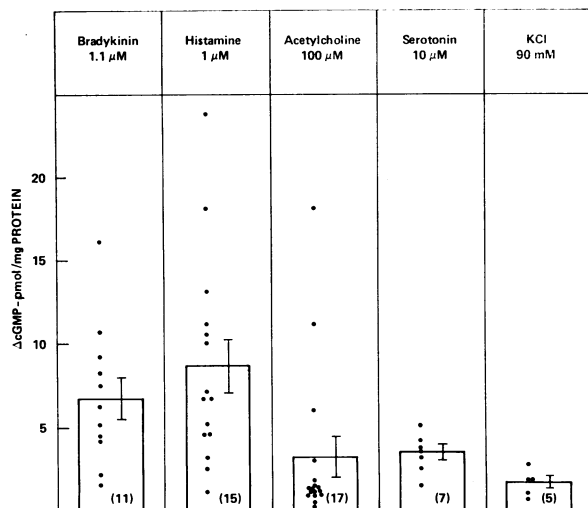


FIGURE 2 Increase in cGMP of umbilical artery above basal levels produced by bradykinin, histamine, acetylcholine, serotonin, and  $\text{K}^+$  ion. Each point represents the mean difference between cGMP content of duplicate samples treated with agonist for 2 min and the cGMP content of segments of the same artery incubated in medium alone. The bars indicate mean  $\pm$  SEM. All experiments except those with  $\text{K}^+$  ion were carried out as described in the legend for Fig. 1. For the latter, segments of artery were incubated for 2 h in 2.5 ml of Krebs-Ringer-Tris medium containing 5 mM  $\text{K}^+$  ion. 5 ml of Krebs-Ringer-Tris medium containing 132 mM  $\text{K}^+$  ion (KCl replaced NaCl) at 37°C was then added to yield a final concentration of 90 mM  $\text{K}^+$  ion and the incubation was terminated 2 min later.

As shown in Fig. 2, when the  $\text{K}^+$  ion concentration of the medium was raised from 5 to 90 mM, significant accumulation of cGMP occurred within 2 min. The effect of  $\text{K}^+$  ion was less than that of bradykinin or histamine and was similar in magnitude to that of acetylcholine or serotonin. Prostaglandins  $\text{E}_2$ ,  $\text{A}_2$ , and  $\text{F}_{2\alpha}$  (5  $\mu\text{g}/\text{ml}$ ) and isoproterenol (1 or 10  $\mu\text{M}$ ) had no effects on the cGMP content of artery segments.

The effect of 100  $\mu\text{M}$  acetylcholine on cGMP accumulation was completely prevented by 1  $\mu\text{M}$  atropine which was without significant effect when added alone or in

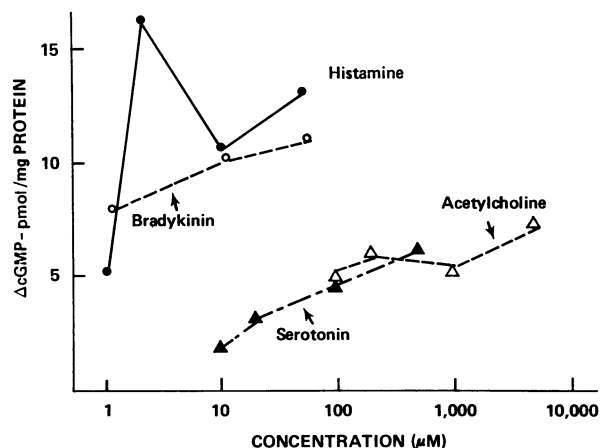


FIGURE 3 Effect of bradykinin, histamine, acetylcholine, and serotonin on the cGMP content in the umbilical artery. Segments of arteries from three cords were pooled and randomly distributed to incubation vials. Segments were incubated for 2 h after which additions of agonists were made and the incubation period terminated 2 min later. Each point represents the mean difference between the cGMP content of duplicate samples treated with agonist for 2 min and the cGMP content of segments incubated in medium alone.

TABLE I  
Effects of Atropine and Pyrilamine on cGMP Accumulation Produced by Acetylcholine, Histamine, Bradykinin, and Serotonin

Agonist concentration	ΔcGMP due to agonist in presence of:*	
	Atropine, 1 μM	Pyrilamine, 0.1 μM
Acetylcholine, 100 μM	12 ± 9 †	66 ± 23
Histamine, 1 μM	111 ± 1	12 ± 7 †
Bradykinin, 1.1 μM	152 ± 57	110 ± 15
Serotonin, 10 μM	75 ± 23	66 ± 10

Samples of tissue were incubated with the specified antagonist for 15 min. The agonist was then added and the incubation terminated 2 min later.

\* Relative to the increment produced by each agonist in the absence of the antagonist = 100. Mean ± SEM,  $n=3$  except for acetylcholine plus pyrilamine where  $n=4$ . The value for each experiment represented the average of duplicate samples from the same umbilical artery.

† Inhibition by the antagonist is significant ( $P < 0.01$ ).

the presence of the other agonists as shown in Table I. 0.1 μM pyrilamine completely abolished the effect of 1 μM histamine (Table I). Pyrilamine did not alter basal cGMP levels nor did it significantly interfere with the effects of acetylcholine, bradykinin, or serotonin. Neither pyrilamine nor atropine interfered with the effect of  $K^+$  on cGMP accumulation (data not shown). Metiamide did not block the effect of 1 μM histamine (Fig. 4). In

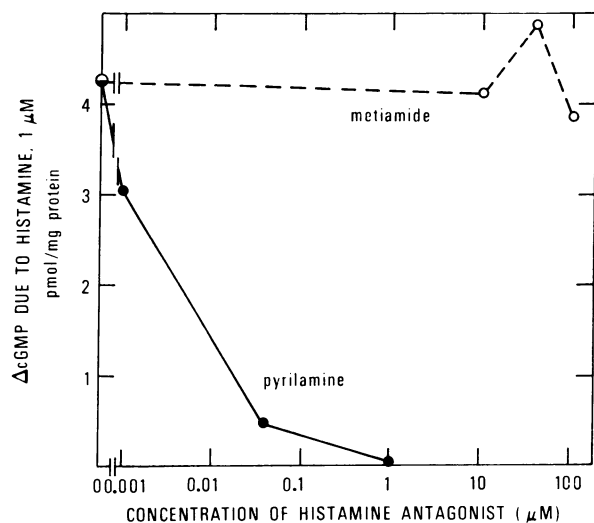


FIGURE 4 Effect of histamine antagonists on cGMP accumulation produced by histamine. Segments of umbilical artery from a single cord were treated as described in Table I. Each point represents the difference between the mean cGMP content of duplicate samples treated with 1 μM histamine plus antagonist at the indicated concentration and that of segments incubated in medium plus antagonist (at the indicated concentration) alone.

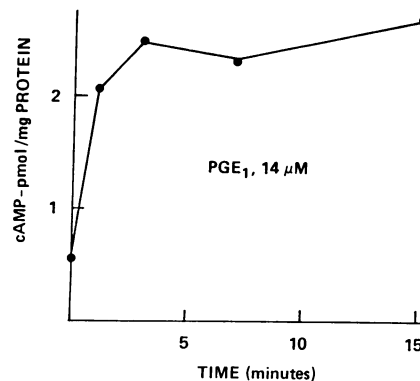


FIGURE 5 Effect of  $PGE_1$  on the time course of cAMP accumulation in human umbilical artery. See Fig. 4 for explanation. Segments of the same artery were exposed to  $PGE_1$  (5 μg/ml), 14 μM for varying periods of time.

fact, at the higher concentrations (50 and 100 μM) metiamide slightly increased basal cGMP content.

The cAMP content of 10 artery preparations incubated for 2 h was  $0.67 \pm 0.09$  pmol/mg protein (mean ± SEM), i.e., lower than the cGMP content of this tissue. In an attempt to magnify the effects of agents that might cause accumulation of cAMP, 1 mM theophylline which did not significantly alter basal levels of cAMP was present in the medium for these experiments. As shown in Fig. 5, after 1 min of exposure of artery segments to 14 μM  $PGE_1$  (5 μg/ml), marked accumulation of cAMP had occurred. The effect of  $PGE_1$  which was apparently maximal at 3 min was maintained for at least 15 min. In six experiments incubation for 3 min with 14 μM  $PGE_1$ , a maximally effective concentration (Fig. 6), increased the cAMP content of the artery

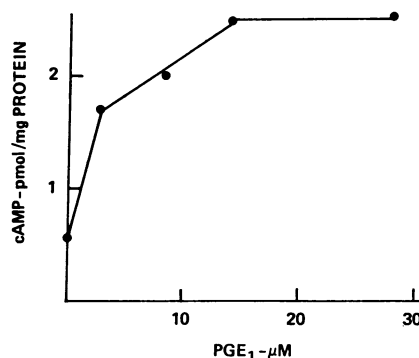


FIGURE 6 Effect of  $PGE_1$  on cAMP content of human umbilical artery. Segments of umbilical artery from a single infant were incubated for 2 h. 15 min before addition of  $PGE_1$ , theophylline was added to a final concentration of 1 mM.  $PGE_1$  was added to the indicated concentration and incubation was terminated 3 min later. Each point represents the mean cAMP content of duplicate samples of segments plus medium.

(plus medium) by  $1.66 \pm 0.45$  pmol/mg protein (mean of paired differences  $\pm$  SEM,  $P < 0.01$ ). 0.5% ethanol, the highest concentration present in incubations with PGE<sub>1</sub>, had no effect.

In other experiments, and in medium containing 1 mM theophylline, isoproterenol (1 or 10  $\mu$ M) as well as 10  $\mu$ M serotonin, 1  $\mu$ M bradykinin, 1  $\mu$ M histamine, 100  $\mu$ M acetylcholine, and 90 mM K<sup>+</sup> ion were without effect on arterial cAMP content.

## DISCUSSION

It has been suggested that cGMP plays a role in smooth muscle contraction and cAMP in relaxation (22). In the umbilical artery, the concentrations of the two cyclic nucleotides appear to be independently controlled in a manner that correlates with and mirrors the contractile behavior of the tissue. Triner, Vulliamoz, Verosky, Habif, and Nahas (23) found that in aortic strips  $\beta$ -adrenergic stimulation produced an increase in cAMP content as well as muscle relaxation. Because of the failure of isoproterenol to cause dilation of umbilical arteries (4, 7) and to cause accumulation of cAMP in our studies, it may be inferred that  $\beta$ -receptors either are lacking in this tissue or, if present, are not functionally linked to adenylate cyclase. Of all of the agents studied, only PGE<sub>1</sub> is reported to cause relaxation of the umbilical artery (9), and, similarly, only PGE<sub>1</sub> caused accumulation of cAMP in this tissue.

The functional significance of the finding that the cGMP content of the term umbilical artery (1 pmol/mg protein) incubated at room air is almost twice that of cAMP remains obscure. In all other mammalian tissues thus far studied, the cAMP concentration is greater than, in some cases 100 times, that of cGMP (22). Perhaps the relatively high cGMP content of the umbilical artery at term and in room air is related to the physiological importance of arterial contraction under these conditions. Umbilical artery constriction can be induced by acetylcholine, histamine, bradykinin, serotonin, and a high concentration of K<sup>+</sup> ion as well as by PGA<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  (6). Of these only PGA<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  failed in our experiments to cause accumulation of cGMP. None of them altered the cAMP content of the tissue. The concentrations of the respective agonists required to produce maximal effects on cGMP are similar to those that have been reported to cause maximal contraction of the artery *in vitro* (4, 6). In several tissues the effect of acetylcholine on cGMP content is apparently mediated through a muscarinic receptor (11-14), and in the umbilical artery it was prevented by atropine, which did not alter the responses to histamine, bradykinin, or serotonin. The effects of histamine but not those of the other agonists were inhibited by pyrilamine. The suggestive (but not statistically significant) inhibition by pyrilamine of the responses to ace-

tylcholine and serotonin may be related in the first case to the anticholinergic properties of most antihistamines and in the second to the observation (24) that effects of serotonin on smooth muscle can be inhibited by antihistamines of the ethylenediamine class, e.g., pyrilamine.

Ash and Schild (25) have postulated at least two types of histamine receptors. Interaction of histamine with H<sub>1</sub> receptors causes vasodilation and smooth muscle contraction, whereas histamine acting through H<sub>2</sub> receptors stimulates gastric acid secretion, elevates heart rate, relaxes uterine smooth muscle and increases the cAMP content of gastric mucosa and leukocytes (26, 27). Pyrilamine, an H<sub>1</sub> antagonist, which abolished the effect of histamine on cGMP accumulation in the umbilical artery, also prevents histamine-induced contraction in this tissue (6). Metiamide, an H<sub>2</sub> antagonist, did not interfere with the effect of 1  $\mu$ M histamine on cGMP in the umbilical artery.<sup>a</sup> Thus it appears that histamine causes accumulation of cGMP in umbilical artery via its interaction with H<sub>1</sub> receptors. There is no evidence that it can, through H<sub>2</sub> receptors, activate adenylate cyclase in this tissue.

Thus in the umbilical artery as in certain other tissues containing smooth muscle, chemical agents that produce contraction also cause accumulation of cGMP and those that induce relaxation increase the concentration of cAMP. PGE<sub>1</sub> presumably increases cAMP in the umbilical artery by causing activation of adenylate cyclase as it does in other tissues. Whether histamine, acetylcholine, bradykinin, serotonin and K<sup>+</sup> ions elevate cGMP as a consequence of increasing (directly or indirectly) synthesis of the nucleotide or decreasing degradation remains to be established.

Because of the cellular heterogeneity of the artery, it is difficult to prove that the changes in cyclic nucleotide content do, in fact, occur in the muscle. For this reason, studies with homogeneous populations of cultured arterial smooth muscle cells, as well as with the umbilical artery, are now in progress to evaluate further the role of cGMP in the contraction produced by acetylcholine, histamine, serotonin, bradykinin, and K<sup>+</sup> as well as by other factors (e.g. oxygen) that have been implicated in the induction of umbilical artery closure at birth.

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<sup>a</sup>The apparent H<sub>2</sub> receptor dissociation constant for metiamide in tissues with known H<sub>2</sub> receptors is  $K_D = 10^{-6}$  M (28).

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