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PROPERTIES OF ADENYLATE CYCLASE AND CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN HAMSTER ISOLATED CAPILLARY PREPARATIONS

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Summary

Capillaries isolated by collagenase digestion of hamster epididymal fat pads were used to examine the properties of endothelial adenylate cyclase and cyclic nucleotide phosphodiesterase. Adenylate cyclase activity in capillary homogenates was increased by 10 μM GTP or 100 μM isoproterenol. Lower concentrations of the catecholamine and 5.7 μM prostagladin E_1 did not stimulate endothelial adenylate cyclase activity unless GTP was included in the assay system. The effects of isoproterenol on capillary adenylate cyclase activity were blocked by propranolol, but were not affected by phentolamine. Phosphodiesterase activity in endothelial homogenates showed anomalous kinetic behavior with either cyclic AMP or cyclic GMP as the enzyme substrate. At substrate concentrations below 1 μM, capillary phosphodiesterase activity hydrolyzed cyclic GMP 2—6 times faster than cyclic AMP. However, at high substrate levels, e.g., 100 μM, cyclic AMP and cyclic GMP were degraded at similar rates. Hydrolysis of 1 μM cyclic AMP by capillary homogenates was stimulated by 0.1 and 1 μM cyclic GMP. Caffeine, 1-methyl-3-isobutylxanthine, papaverine and dipyridamole SQ 20009 were effective inhibitors of capillary phosphodiesterase activity. In contrast, imidazole enhanced the activity of the enzyme. The presence of adenylate cyclase and phosphodiesterase activities in hamster isolated capillaries is consistent with a role for cyclic AMP in the regulation of endothelial function. Moreover, the experiments described here indicate that hamster isolated capillaries are useful model systems for studying the metabolism of vascular endothelium.

Introduction

With the development of techniques for obtaining endothelial cultures [1,2] and relatively homogeneous suspensions of isolated capillaries [3], there has been renewed interest in the biochemistry of vascular endothelium. Metabolic studies on endothelial cells have attracted considerable attention in recent years because there is evidence which suggests that changes in endothelial function may be important in thrombus formation [4], degenerative vascular disease [5], and diabetic angiopathies [6]. As a result of experiments with cultured endothelial cells and capillary preparations it has become apparent that vascular endothelium, which was once considered a passive tissue, is capable of synthesizing prostaglandins [7,8], sulfated glycosaminoglycans [9], von Willebrand factor [10], and angiotensin-converting enzyme [11]. In addition, vascular endothelium responds to vasoactive hormones, e.g., catecholamines, histamine, and prostaglandins, with increases in intracellular cyclic nucleotide levels [3,12]. The latter observations seem to indicate that cyclic AMP and/or cyclic GMP could be involved in the regulation of endothelial processes. The work described in this report offers further support for this hypothesis by demonstrating that both cyclic nucleotide phosphodiesterase activity and a hormonally responsive adenylate cyclase are characteristic of hamster capillary endothelium. A preliminary account of some of the data presented here has been published elsewhere [13].

Experimental procedures

Preparation of isolated capillaries. With the exception of the collagenase digestion step, all manipulations were carried out at room temperature in order to avoid interference by congealed lipids. Epididymal fat pads were removed from decapitated male hamsters, and were minced and suspended in plastic vials (approx. two pads/vial) with 3 ml of modified Krebs-Ringer phosphate buffer (pH 7.4) containing no added calcium, 5.5 mM D-glucose, and 3% bovine serum albumin. 1.7 mg/ml crude collagenase was added for digestion and the vials were shaken vigorously on a Dubnoff-type water bath at 37°C. Under these conditions, tissue disaggregation was essentially complete within 5 min. Adipose tissue digests were diluted with 4 ml of the phosphate buffer and forced through silk into a plastic centrifuge tube. The volume of the pooled digest was increased to 40 ml before the preparation was centrifuged at $32 \times g$ for 2 min. A small pellet consisting primarily of pieces of large blood vessels was discarded. Adipocytes and the supernatant fraction were mixed by gentle inversion and the resulting fat cell suspension was centrifuged at $800 \times g$ for 2 min. The endothelial pellet fraction was dispersed in the modified Krebs-Ringer phosphate buffer and the capillary suspension was centrifuged at $80 \times g$ for 2 min. The $80 \times g$ capillary pellet fraction was resuspended in 15 ml of phosphate buffer and filtered through 74 μm nylon mesh. Endothelial networks were retained on the nylon while blood elements and occasional free stromal cells passed through the pores in the material. The capillary tufts were removed from the mesh with a stream of the modified Krebs-Ringer phosphate buffer from a syringe, resuspended in the same buffer, and collected by cen-

trifugation at 800 × *g* for 2 min. This final capillary pellet fraction was dispersed in a convenient amount of an appropriate incubation or homogenization buffer by slow passage through a siliconized Pasteur pipet. Approx. 1 mg dry weight of capillary endothelium was obtained from each hamster.

Biochemical analyses. Endothelial CO_2 production from glucose was estimated as described by Schimmel [14]. For these studies, isolated capillaries were suspended in a modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing no added calcium, 0.5 mM D-glucose and 4% bovine serum albumin. Rubber caps were used to seal 1 ml aliquots of the capillary preparations in plastic vials containing 0.05 μCi D-[^{14}C]glucose/ml. Capillaries were incubated at 37°C for 0.5—2 h before being separated from the buffer solution by filtration through glass wool. The amount of glucose which remained in the bicarbonate buffer at the end of the incubation period was measured using a Technicon AutoAnalyzer.

For adenylate cyclase experiments, capillary preparations were homogenized with five strokes of a Pyrex 7727 ground glass tissue grinder (7 ml capacity) in cold 10 mM Tris-HCl buffer (pH 7.4) supplemented with 1 mM dithiothreitol. In some instances, 1 mM $MgCl_2$ was included in the homogenization buffer. The presence of Mg^{2+} during capillary homogenizations appeared to have no effect on endothelial adenylate cyclase activity which was determined using the method of Salomon et al. [15]. The reaction mixture contained capillary homogenate (75—100 μg dry weight), 10 mM Tris-HCl (pH 7.4), 12 mM $MgCl_2$, 2 mM dithiothreitol, 16 mM creatine phosphate, 64 U/ml creatine phosphokinase, 2 mM ATP, 1 mM cyclic AMP, 0.5 μCi [α-^{32}P]ATP, and hormones or other agents in a total volume of 100 μl. Assays were stopped by the addition of 0.5 ml of a solution of 2.5% trichloroacetic acid, 0.07 mM cyclic AMP, and approx. 10 000 dpm of cyclic [^{3}H]AMP to follow recovery (45—65%) of the cyclic nucleotide. Samples were centrifuged at 800 × *g* for 10 min to remove precipitated proteins. Cyclic [^{32}P]AMP was isolated from supernatant fractions by sequential chromatography on Dowex 50 × 4 (100—200 mesh) and neutral alumina. Radioactivity in column eluates was quantified with a liquid scintillation counter using tT-21 as the fluor [16].

For phosphodiesterase studies, capillaries were homogenized with ten strokes of a Pyrex 7727 ground glass tissue grinder (7 ml capacity) in cold 60 mM Tris-HCl buffer (pH 7.4) containing 1 mM $MgSO_4$ and 1 mM β-mercaptoethanol. Phosphodiesterase activity in endothelial homogenates was assayed using a radioisotope method [17]. Cyclic AMP (0.025—400 μM) or cyclic GMP (0.025—100 μM) was used as the phosphodiesterase substrate. Inhibitors and other agents were included in the assay system as required. The assay depended on the conversion of cyclic [^{3}H]AMP or cyclic [^{3}H]GMP to [^{3}H]-adenosine or [^{3}H]guanosine, respectively, through sequential reactions catalyzed by phosphodiesterase and *Crotalus atrox* venom. Tritiated nucleosides were isolated by column chromatography on Dowex 1-X2 (200—400 mesh). Recovery (85—95%) of [^{3}H]adenosine or [^{3}H]guanosine from anion-exchange columns was monitored with [^{14}C]adenosine or [^{14}C]guanosine, respectively. Radioactivity in column eluates was estimated with a liquid scintillation counter and tT-21 [16] as the fluor. Enzyme dilutions were chosen to insure that 3—20% of the substrate was hydrolyzed during the 30 min assay

period. Under these conditions, adenosine or guanosine formation was linear with respect to time and to enzyme concentration. Only fresh capillary homogenates were assayed for phosphodiesterase as the activity of the enzyme in these preparations decreased markedly with freezing and thawing.

All biochemical assays were performed in duplicate or triplicate and corrected for recoveries. Data were expressed as milligrams of dry weight [18] because exposure of capillaries to albumin and collagenase during the isolation procedure appeared to interfere with protein determinations. Washing isolated capillaries with albumin-free buffer was avoided since it tended to reduce the final yields of vascular endothelium.

Materials. Male hamsters (*Mesocricetus auratus*, 80—100 g) were obtained from Charles River-Lakeview, North Wilmington, MA; crude collagenase (type CLS) from the Worthington Biochemical Co., Freehold, NJ; bovine serum albumin fraction V from the Armour Pharmaceutical Co., Chicago, IL; *C. atrox* venom from the Ross Allen Reptile Institute, Inc., Silver Springs, FL; papaverine, Dowex 1-X2 (200—400 mesh), and Dowex 50 × 4 (100—200 mesh) from the Sigma Chemical Co., St. Louis, MO; ATP and GTP from Boehringer-Mannheim Biochemicals, Indianapolis, IN; 1-methyl-3-isobutylxanthine from the Aldrich Chemical Co., Milwaukee, WI; and cyclic [G-^{3}H]AMP (20—50 Ci/mmol), cyclic [G-^{3}H]GMP (5—10 Ci/mmol), [8-^{14}C]adenosine (40—60 Ci/mol), [8-^{14}C]guanosine (greater than 400 Ci/mol), [α-^{32}P]ATP (10—30 Ci/mmol), and D-[U-^{14}C]glucose (1—5 Ci/mol) from New England Nuclear, Boston, MA. Tritiated cyclic AMP and cyclic GMP were purified on Dowex 1-X2 (200—400 mesh) before use. Prostaglandin E_1 was generously provided by Dr. John Pike of the Upjohn Co., Kalamazoo, MI. Isoproterenol was kindly supplied by Dr. F.C. Nachod of the Sterling-Winthrop Research Institute, Rensselaer, NY. RO 20-1724 (4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone) and dipyridamole were obtained from Dr. Herbert Sheppard of Hoffmann-LaRoche, Inc., Nutley, NJ, U.S.A. and from Dr. Hasso Schroeder, Thomas GmbH., Stuttgart, F.R.G., respectively. SQ 20009 (1-ethyl-4-[isopropylidenehydrazino]-1H-pyrazolo-[3,4-*b*]-pyridine-5-carboxylic acid, ethyl ester, hydrochloride) was gift from Dr. Sidney M. Hess of the Squibb Institute for Medical Research, New Brunswick, NJ.

Results

Homogeneity of capillary preparations

In order to obtain adequate capillary yields, Wagner et al. [3] incubated rat epididymal fat pads at 37°C for 45 min with approximately 7 mg of crude collagenase/ml of physiological buffer. Since it was possible that such extended exposure to proteolytic enzymes could disrupt endothelial plasma membranes and their component hormone receptors, the methodology of Wagner et al. [3] was modified to prepare capillary endothelium from hamster epididymal fat pads. At 37°C hamster adipose tissue is disaggregated by low concentrations of crude collagenase in less than 5 min.

Phase-contrast microscopy showed that contamination of hamster capillary suspensions by blood cells and stromal elements was minimal. The omission of calcium from isolation and incubation media appeared to prevent hamster

capillaries from adhering together in large clusters. Approx. 80% of the endothelial cells in hamster capillary segments excluded trypan blue and were, by this criterion, judged to be viable [19]. Capillaries removed 4.2 ± 1.3 [3] nmol of glucose/mg dry weight per h from the media and converted 0.23 ± 0.01 [11] nmol of glucose/mg dry weight per h to CO_2. Rasio [20] has reported a similar pattern of glucose metabolism for rat isolated capillary preparations.

Capillary adenylate cyclase activity

Under the assay conditions used in this study, adenylate cyclase activity in endothelial homogenates was linear for only 8 min. When 10 μM GTP was included in the reaction mixture, capillary adenylate cyclase activity increased 2—5-fold as compared to the basal value. However, the rate of cyclic AMP formation by GTP-stimulated endothelial adenylate cyclase also fell markedly after 8 min. Since an ATP-regenerating system was incorporated into the adenylate cyclase assay system, it seemed unlikely that the time-dependent decrease in enzyme activity was a function of substrate depletion. It was nevertheless possible that capillary adenylate cyclase was unstable in the crude endothelial homogenates. Incubation periods for subsequent adenylate cyclase determinations were therefore limited to 5 min. Both basal and GTP-stimulated endothelial adenylate cyclase activities were linear with respect to enzyme concentration at this time.

The data presented in Table I demonstrated that GTP, in addition to increasing capillary adenylate cyclase activity, was required for activation of the enzyme by 1 μM and 10 μM isoproterenol. A significant stimulation of endothelial adenylate cyclase activity was observed in response to 100 μM isoproterenol alone, but GTP acted synergistically with this concentration of the catecholamine to further elevate enzyme activity. The increase in capillary adenylate cyclase activity produced by 10 μM isoproterenol was blocked by propranolol, a β-adrenergic receptor antagonist, but was not affected by the

TABLE I

CAPILLARY ADENYLATE CYCLASE ACTIVITY IN THE PRESENCE OF GTP AND ISOPROTERENOL

Hamster isolated capillaries were homogenized in cold 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM dithiothreitol. The homogenates were assayed immediately for adenylate cyclase activity. Incubations were for 5 min. Results are the means ± S.E. for determinations on three different preparations.

Additions	Adenylate cyclase activity (pmol cyclic AMP/mg dry weight)
None	56.1 ± 9.4
10 μM GTP	110 ± 10 *
1 μM isoproterenol	53.4 ± 6.2
10 μM isoproterenol	66.6 ± 7.8
100 μM isoproterenol	124 ± 47 *
10 μM GTP + 1 μM isoproterenol	205 ± 20 *,**
10 μM GTP + 10 μM isoproterenol	276 ± 16 *,**
10 μM GTP + 100 μM isoproterenol	281 ± 9 *,**

* Significantly different from basal, $P < 0.05$.

** GTP + isoproterenol significantly different from GTP, $P < 0.05$.

α-adrenergic receptor antagonist phentolamine. In preliminary experiments, 30 μM epinephrine in combination with 10 μM GTP also caused a rise in endothelial adenylate cyclase activity which was blocked by 1 μM propranolol, but not by 1 μM phentolamine. Since neither propranolol nor phentolamine interfered with the ability of GTP alone to stimulate capillary adenylate cyclase activity, it appeared that the effects of the guanine nucleotide could not be attributed to possible sympathetic nerve contaminants in the capillary suspensions. Buonassisi and Venter [12] have previously reported that 10 μM propranolol decreased cyclic AMP levels in endothelial cultures exposed to 10 μM norepinephrine while phentolamine antagonized catecholamine-mediated increases in endothelial cyclic GMP accumulation.

Like low concentrations of isoproterenol, 5.7 μM prostaglandin E_1 had no marked effect on capillary homogenate adenylate cyclase activity unless GTP was included in the reaction mixture. In the presence of 10 μM GTP, the prostaglandin elevated endothelial adenylate cyclase activity in a concentration-dependent manner (Fig. 1). The observation that GTP was crucial to prostaglandin as well as catecholamine stimulation of hamster capillary adenylate cyclase activity suggested that the rat capillary enzyme showed only minimal hormone responsiveness because the guanine nucleotide was not included in the assay system [3]. Results of studies with capillaries which had been homogenized in a buffer containing 5 μg/ml indomethacin appeared to indicate that the ability of GTP to increase endothelial adenylate cyclase activity was independent of newly synthesized prostaglandins. In view of a recent report by Wallach et al. [21], it is conceivable that the GTP-produced increases in capillary adenylate cyclase activity were related to exposing endothelial cells to proteolytic enzymes during the isolation procedure.

Kinetic properties of capillary phosphodiesterase

Cyclic nucleotide phosphodiesterase activity was determined in capillary homogenates using cyclic AMP or cyclic GMP as the enzyme substrate (Fig. 2). Graphic analysis of the assay results revealed anomalous kinetics for capillary

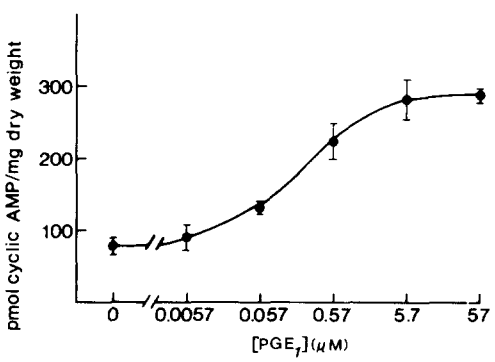

Fig. 1. Capillary adenylate cyclase activity in response to increasing concentrations of prostaglandin E_1 (PGE_1). Hamster isolated capillaries were homogenized in cold 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM dithiothreitol. The homogenates were assayed immediately for adenylate cyclase activity in the presence of 10 μM GTP. Incubations were for 5 min. Results are the means ± S.D. for three or four determinations and are representative of data from three different preparations.

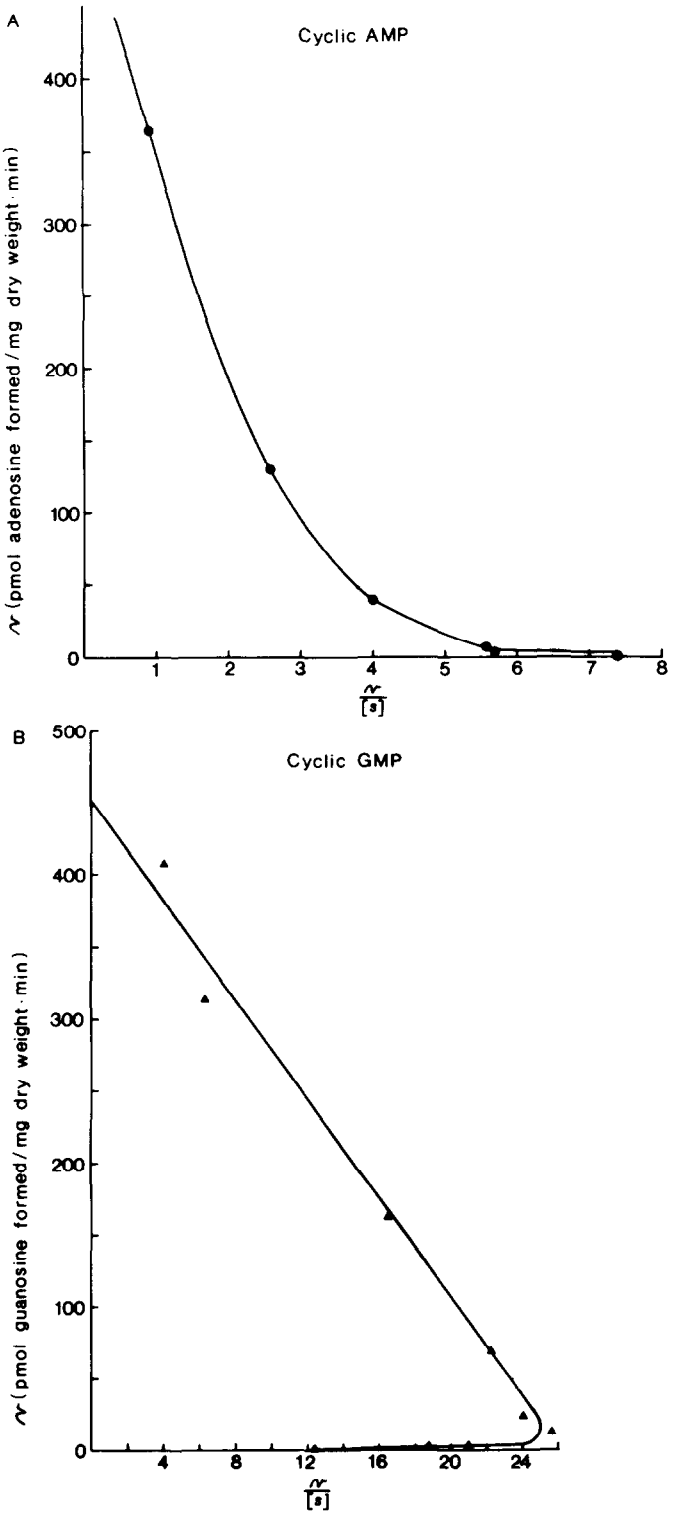

Fig. 2. Kinetic behavior of endothelial cyclic nucleotide phosphodiesterase. Hamster isolated capillaries were homogenized in cold 60 mM Tris-HCl buffer (pH 7.4) containing 1 mM β-mercaptoethanol and 1 mM $MgSO_4$. The homogenates were assayed immediately for phosphodiesterase activity using 0.05—400 μM cyclic AMP (panel A) and 0.025—100 μM cyclic GMP (panel B) as the substrate for the enzyme. Results are the means of two determinations and are representative of data from eight different preparations

phosphodiesterase when cyclic AMP was the substrate. With cyclic GMP as the substrate for endothelial phosphodiesterase, the Eadie-Hofstee plot was concave downward at low concentrations of the nucleotide. This kinetic pattern was similar to that observed for cyclic GMP phosphodiesterase activity in $(NH_4)_2SO_4$ fractions from rat livers [22]. At substrate concentrations below 1 μM, capillary phosphodiesterase hydrolyzed cyclic GMP 2—6 times faster than cyclic AMP. In contrast, at high substrate levels, e.g. 100 μM, cyclic AMP and cyclic GMP were degraded at similar rates. Linear regression analysis of the straight segment of the kinetic plot shown in Fig. 2 (0.5—100 μM cyclic GMP; correlation coefficient = 0.99) revealed an apparent K_m for cyclic GMP of 17.5 μM and a V of 453 pmol guanosine formed/mg dry weight per min.

The non-linear Eadie-Hofstee plots shown in Fig. 2 were compatible with the existence of multiple phosphodiesterase activities in capillary homogenates. However, partial purification of endothelial phosphodiesterase activities was not attempted in view of the very limited quantities of capillary endothelium which could be isolated from the epididymal fat pads of a single hamster. Additional information about the phosphodiesterase activities in capillary homogenates was obtained by examining cyclic AMP hydrolysis in the presence of cyclic GMP and vice versa. These studies indicated that the capillary preparations contained cyclic AMP phosphodiesterase activity which could be stimulated by 0.1 and 1 μM cyclic GMP.

TABLE II

EFFECTS OF VARIOUS AGENTS ON CAPILLARY PHOSPHODIESTERASE ACTIVITY

Hamster isolated capillaries were homogenized in cold 60 mM Tris-HCl Buffer (pH 7.4) containing 1 mM β-mercaptoethanol and 1 mM $MgSO_4$. The homogenates were assayed immediately for phosphodiesterase activity. The enzyme/substrate mixtures contained cyclic AMP or cyclic GMP plus 50 μM theophylline, 50 μM 1-methyl-3-isobutylxanthine, 50 μM SQ 20009, 50 μM papaverine, 50 μM dipyridamole, 50 μM RO 20-1724 or 40 mM imidazole. At the beginning of the incubation period, the pH of the assay system in the presence of 40 mM imidazole was approx. 7.7. Values are means ± S.E. for determinations on 3—10 different capilllary homogenates.

Addition to assay	Phosphodiesterase activity			
	pmol adenosine formed/mg dry weight per min		pmol guanosine formed/mg dry weight per min	
	1 μM cyclic AMP	100 μM cyclic AMP	1 μM cyclic GMP	100 μM cyclic GMP
None	7.50 ± 1.33	374 ± 55	23.2 ± 2.9	473 ± 28
Theophylline	3.66 ± 1.13	227 ± 61	13.3 ± 2.5 **	436 ± 31
1-Methyl-3-isobutylxanthine	4.15 ± 0.74	332 ± 46	5.02 ± 0.70 ***	251 ± 26 ***
SQ 20009	2.48 ± 0.88 *	194 ± 56	10.2 ± 2.0 **	366 ± 22 *
Papaverine	1.58 ± 0.48 **	112 ± 33 **	11.3 ± 4.6 *	286 ± 28 ***
Dipyridamole	2.43 ± 0.77 *	132 ± 40 **	7.50 ± 1.28 ***	216 ± 20 ***
RO 20-1724	6.10 ± 0.39	506 ± 97	16.6 ± 3.6	419 ± 25
Imidazole	10.5 ± 0.99	648 ± 101 *	14.0 ± 2.2	570 ± 29 *

* Significantly different from control, $P < 0.05$.
** Significantly different from control, $P < 0.02$.
*** Significantly different from control, $P < 0.01$.

Inhibition of capillary phosphodiesterase activity

Caffeine produced a concentration-dependent inhibition of endothelial phosphodiesterase activity when either cyclic AMP or cyclic GMP was the substrate for the enzyme. Caffeine-induced decreases in capillary phosphodiesterase activity were similar at 1 and 100 μM cyclic AMP. However, the xanthine was a more potent inhibitor at low rather than at high cyclic GMP levels. Furthermore, cyclic GMP hydrolysis by capillary phosphodiesterase was more sensitive to caffeine inhibition than was cyclic AMP hydrolysis.

Phosphodiesterase activity in capillary homogenates was also significantly decreased in the presence of 50 μM concentrations of 1-methyl-3-isobutylxanthine, SQ 20009, papaverine, and dipyridamole (Table II). 1-Methyl-3-isobutylxanthine, like caffeine, appeared to be a more potent inhibitor of cyclic GMP hydrolysis than of cyclic AMP hydrolysis. 50 μM theophylline caused capillary phosphodiesterase activity to fall by 43% when 1 μM cyclic GMP was the substrate. 50 μM RO 20-1724 had no marked effect on endothelial phosphodiesterase activity with either substrate. The addition of 40 mM imidazole to the enzyme assay system produced a substantial increase in rate of hydrolysis of 100 μM, but not 1 μM cyclic AMP or cyclic GMP. Other investigators have also observed that imidazole stimulated phosphodiesterase activity at high, but not at low substrate concentrations [23—25]. The stimulatory effect of imidazole on phosphodiesterase activity was originally reported by Butcher and Sutherland [26] who used bovine heart as the source of the enzyme and assayed at high substrate levels. Neither imidazole nor any of the other agents used in this series of experiments interfered with the snake venom-catalyzed step in the phosphodiesterase assay.

Discussion

Endothelial cell cultures have become popular preparations for investigating the metabolism of vascular endothelium [1,2,7—12]. However, the possibility that normal endothelial cell function may be perturbed in the course of adaptation to in vitro conditions must be considered when interpreting the results of experiments using cultured endothelium. In addition, cultured endothelial cells appear to show a tendency toward precocious senescence [27—29]. For these reasons, freshly isolated capillaries were used to examine the properties of endothelial adenylate cyclase and cyclic nucleotide phosphodiesterase. The choice of hamster adipose tissue as the source of capillaries allowed us to obtain endothelial segments which had received only minimal exposure to proteolytic enzymes which could disrupt plasma membranes and the adenylate cyclase system.

The data presented in this report complement earlier studies which quantified the effects of hormones on endothelial cyclic AMP and cyclic GMP levels [3,12]. Hamster isolated capillaries were found to have cyclic nucleotide phosphodiesterase activity and a hormonally responsive adenylate cyclase which shared several characteristics with the corresponding enzymes from other tissues. For example, capillary adenylate cyclase, like a number of non-endothelial adenylate cyclases [30], required GTP for hormonal activation. In addition, the kinetic behavior and inhibitor sensitivities of capillary homogenate

phosphodiesterase were similar to those of other crude preparations of the enzyme [31]. Furthermore, vascular endothelium, like many other tissues [22,32—35] appeared to contain phosphodiesterase activity which could be stimulated by micromolar quantities of cyclic GMP at low substrate levels of cyclic AMP.

The presence of adenylate cyclase and cyclic nucleotide phosphodiesterase activities in hamster-isolated capillaries points to a role for cyclic AMP, and possibly cyclic GMP, in the regulation of endothelial function. It has been suggested that the movement of nutrients, waste products, and atherogenic substances through vascular endothelium could involve transport across endothelial plasma membranes in conjunction with endothelial cell contraction [36, 37]. There is already evidence indicating that changes in the permeability of some epithelial membranes to water, sodium, and small molecules such as urea may be mediated by altered intracellular cyclic AMP levels [38,39]. Since vascular endothelium and smooth muscle have several morphological features in common, e.g., endothelial cells contain a protein which is immunologically similar to uterine smooth muscle actomyosin [40], it is conceivable that contraction of the two cell types depend on similar mechanisms. Cyclic nucleotides have been proposed as mediators of the contractile state of smooth muscle, but this idea remains highly controversial [41,42]. The finding that glycosaminoglycan production was enhanced by hormones which elevate cyclic AMP levels in mouse fibroblasts [43] raises the possibility that cyclic nucleotides may also influence glycosaminoglycan synthesis in vascular endothelium.

The experiments described in this paper were primarily undertaken to determine the fundamental properties of endothelial adenylate cyclase and cyclic nucleotide phosphodiesterase. However, they also reinforce claims that isolated capillaries are useful model systems for studying the biochemistry of vascular endothelium [3,20,44—46]. Isolated capillary preparations could be especially valuable in clarifying the etiology of some microvascular diseases. In particular, work with capillaries from animals with diabetes mellitus might provide much needed insight into the pathogenesis of diabetic angiopathies.

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