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STIMULATION OF PLACENTAL PRORENIN SECRETION BY SELECTIVE INHIBITION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES

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Abstract—Prorenin secretion by human villous placenta is known to be stimulated by activation of adenylate cyclase and enhanced cyclic AMP (cAMP) generation. Placental tissue contains predominantly type III (cGMP-inhibited) and type IV (cAMP-specific) phosphodiesterases (PDEs), which inactivate cAMP. To evaluate the role of PDE subtypes in the regulation of prorenin secretion by human placenta, explants were cultured in the presence of isobutylmethylxanthine (IBMX), a non-selective PDE inhibitor, and selective inhibitors for various PDE subtypes. Inhibition of PDE subtypes with cilostamide (type III), Ro 20-1724 (type IV) and zardaverine (types III and IV) increased prorenin release. Inhibition of type I (Ca^{2+}/calmodulin-dependent) PDE by 8-MeoM-IBMX and of type V (cGMP-specific) PDE by zaprinast or dipyridamole did not affect prorenin secretion. The stimulation of prorenin secretion by PDE inhibitors was attenuated by cAMP-dependent protein kinase inhibition. The selective PDE inhibitors caused a parallel increase in media cAMP and prorenin and also increased tissue prorenin levels. These studies demonstrate that cAMP degradation by type III and IV PDE isoenzymes is a major regulatory mechanism for placental prorenin secretion. It is suggested that enhancers of adenylate cyclase activity are constitutively present in placenta and influence prorenin synthesis and release.

Key words: renin–angiotensin system; tissue culture; phosphodiesterase; placenta; gonadotropin; intracellular signaling

RAS† components have been found in human villous placenta, although the function and regulation of RAS activity in this tissue have not been described completely [1]. Suggested physiological roles of the placental RAS include circulatory regulation and autocrine/paracrine signaling effects on hormone secretion [2–4]. The development of the placental vascular bed may also be influenced by RAS activity, as demonstrated by the angiogenic properties of angiotensin II in other tissues [5–7]. A number of studies have investigated the regulatory mechanisms that influence the secretion of the precursor to the rate-limiting enzyme of this system, prorenin, by human reproductive tissues including the ovary [8], testis [9], chorion and decidua [10, 11]. In these studies, contrasts in the second messenger signaling mechanisms for prorenin secretion between the kidney and reproductive tissues were found.

Secretion of prorenin by human placenta has been shown to be stimulated by activators of adenylate cyclase, such as forskolin and cholera toxin [12]. Activation of β-adrenoceptors evokes prorenin secretion by human placental explant cultures, and this response is potentiated by non-selective PDE inhibition [13].

To further characterize the role of PDE and cAMP signaling in mediating placental prorenin secretion, the following studies were conducted. Human placental explants were incubated in the presence of PDE inhibitors, and prorenin concentrations in the media were measured. For comparative purposes, effects of PDE inhibition on the secretion of the placental hormone, hCG, were evaluated. To determine if the effects of PDE inhibition were mediated by cAMP, culture medium concentrations of this nucleotide were determined. In addition, we tested the hypothesis that PDE inhibitor effects on prorenin secretion are mediated by cAMP-dependent protein kinase activation.

MATERIALS AND METHODS

Materials and solutions. Ro 20-1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidone] was purchased from Biomol Research (Plymouth, MA). 8-MeoM-IBMX was a gift from Dr. Jack N. Wells (Vanderbilt University, Nashville, TN). Cilostamide [*N*-cyclohexyl-*N*-methyl-4-(1,2-dihydro-2-oxo-6-quinohyloxyl)-butyramide; OPC-3639] was a gift from Otsuka Pharmaceutical (Osaka, Japan). M&B 22,948 (zaprinast; 2-*O*-propoxyphenyl-8-azapurin-6-one) was a gift from Rhone-Poulenc-Rorer (Essex, U.K.).

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† Abbreviations: RAS, renin–angiotensin system; cAMP, cyclic AMP; PDE, phosphodiesterase; IBMX, isobutylmethylxanthine; 8-MeoM-IBMX, 8-methoxy-methyl-isobutylmethylxanthine; and hCG, human chorionic gonadotropin.

Table 1. Classification of phosphodiesterase (PDE) inhibitors

Compound	PDE (primary target)	IC_{50} (μM)	Concentration used in study (μM)	Ref.
3-Isobutylmethylxanthine (IBMX)	Non-selective	5–10	0.5–1000	15
8-Methoxymethyl-3-isobutylmethylxanthine (8-MeoM-IBMX)	Type I Ca^{2+}/calmodulin-dependent	4–5	1–100	16
Cilostamide	Type III cGMP-inhibited	0.005–0.05	0.1–500	17
Ro 20-1724	Type IV cAMP-specific	2–9	5–500	18
Zardaverine	Types III and IV	0.1–0.8	0.1–1000	19
Dipyridamole	Type V cGMP-specific	0.9	0.4–40	20
Zaprinast	Type V cGMP-specific	4–18	3–500	21

Classification scheme is based upon that proposed by Beavo and Reifsnyder [14].

Zardaverine [6-(4-difluoromethoxy-3-methoxyphenyl)-3(2*H*)pyridazinone] was donated by Byk Gulden Pharmaceuticals (Konstanz, Germany). Terbutaline, IBMX and dipyridamole were purchased from the Sigma Chemical Co. (St. Louis, MO). [^{125}I]Angiotensin I was purchased from DuPont-New England Nuclear (Boston, MA) and [^{125}I]β-hCG was obtained from Binax (Portland, ME). Stock solutions of each compound were freshly prepared before addition to the incubation medium.

Phosphodiesterase inhibition. PDE inhibitors are classified by their inherent selectivity for "families" of PDE isoforms, which are described by nucleotide specificity [14]. In the present study, a representative compound was selected for each family based upon the highest degree of selectivity (Table 1). In addition, zardaverine, with high selectivity for type III and IV PDEs and a non-selective PDE inhibitor, IBMX, were used to evaluate the effects of inhibiting multiple PDE isoforms on prorenin secretion.

Placental explant culture. Human placentas were obtained from term pregnancies in which no complications were noted. Placental explants were prepared for incubation in culture medium within 20 min of delivery in accordance with the method of Huot *et al.* [22]. First, the fetal membranes were removed, and then 2 × 2 cm portions were dissected from the villous placenta. Each portion was rinsed briefly in culture medium, and slices (5 mm in thickness) were prepared using a Stadie–Riggs microtome. One slice was placed in each 22-mm well of a 24-well plate. Tissues were incubated with 2 mL of CMRL 1066 medium with 0.1% bovine serum albumin in 95% air and 5% CO_2 at 37°. The medium was changed at 2 and 24 hr, and the experiment was terminated at 48 hr.

At the completion of the experiment, tissues were sonicated in 1 mL of 5 mM EDTA for 15 sec before centrifugation at 5000 *g* for 20 min. The supernatant was collected and stored at −20°, as was the medium, until the time of assays. Each experiment was conducted with at least three separate placentas, and a minimum of three replicates was used for the same treatment from each placenta.

Renin assay. Total renin was determined after trypsin activation of samples by radioimmunoassay of angiotensin I generated from sheep substrate as previously described [10, 11]. Briefly, prorenin was converted to active renin by limited proteolysis with 2 μg/mL bovine trypsin at room temperature for 30 min. The reaction was then stopped with the addition of 50 μg/mL soybean trypsin inhibitor. The enzymatic assay of renin was carried out in the presence of 5 mM EDTA and sheep renin substrate at 37° for 1 hr. Radioimmunoassay for angiotensin I generation by active renin was performed similarly in the absence of trypsin. Prorenin values were determined as the difference between total and active renin. Human renin obtained from the National Institute for Biological Standards (London, U.K.) was used as a standard.

Chorionic gonadotropin assay. The hCG concentrations in media were determined by radioimmunoassay of the β-subunit with [^{125}I]β-hCG (Binax) and hCG antibody [anti-β-hCG SB6 (rabbit), National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD]. The antigen–antibody complex was precipitated with *Staphylococcus aureus* cell suspension (Pansorbin, Catalog No. 507858, Calbiochem, La Jolla, CA) and centrifuged at 3000 *g* for 20 min. Standards curves were performed for each assay using hCG standard [(CR-127), National Institute of Child Health and Human Development, National Institutes of Health] over a concentration range of 4.6 to 2400 mIU/mL. Samples were assayed in duplicate, and the results expressed as the mean of the two.

Cyclic AMP assay. A radioimmunoassay was used to measure cAMP (Incstar, Stillwater, MN). Samples were assayed with reference to a standard

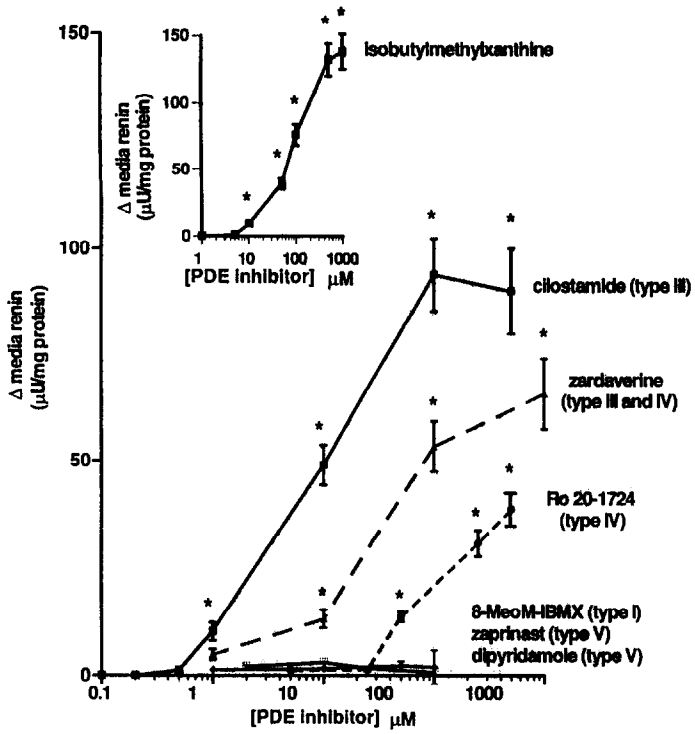

Fig. 1. Effects of phosphodiesterase inhibitors on prorenin secretion by human placental explants. Placental explants were incubated in the presence of selective inhibitors from each family of PDE isozymes for 48 hr. Values represent the means ± SEM for a change in concentrations of prorenin in media relative to controls (5.3 ± 0.4 μU/mg protein). Prorenin concentrations were measured from a minimum of 4 separate placentas with 6 replicates. The effect of non-selective PDE inhibition by isobutylmethylxanthine (IBMX) on prorenin is shown in the inset. Key: (*) significant difference from control, $P < 0.05$.

concentration range of 0.0156 to 2.0 nmol/L. Maximum sensitivity was 0.02 nmol/L.

Protein determination. Soluble protein in placental homogenates was assayed by the method of Bradford [23], using bovine serum albumin as a standard.

Statistical analysis. As multiple studies have demonstrated that the vast majority (> 90%) of total renin present is in the form of prorenin, the data presented are medium and tissue concentrations of this form of renin. Medium concentrations of prorenin (μU/mL), hCG (mIU/mL) and cAMP (pmol/mL) are expressed in terms of tissue protein concentrations (mg/mL), and values are expressed as means ± SEM. In each case, results were evaluated between groups by analysis of variance for repeated measures and two-tailed Student's test whenever appropriate, with significance determined at the level of $P < 0.05$.

RESULTS

Concentration–response effects of phosphodiesterase inhibitors on prorenin secretion by human placental explants. The first studies addressed the concentration–response effects of representative inhibitors from each PDE family on placental explant prorenin secretion. Placental prorenin secretion was enhanced 8- to 20-fold from a basal level of 5.3 ± 0.4 μU/mg protein after 48 hr of incubation with PDE inhibitors (Fig. 1). The relative order of potencies for this was: cilostamide (type III) > zardaverine (types III and IV) > Ro 20-1724 (type IV). No stimulation was observed with 8-MeoM-IBMX (type I), dipyridamole (type V) or zaprinast (type V). Non-selective inhibition by IBMX stimulated prorenin secretion from 5 to 125 μU/mg protein in a concentration-dependent fashion. The maximum response exceeded that of any of the selective inhibitors. Tissue concentrations of prorenin in response to incubation with PDE inhibitors for 48 hr showed a similar pattern to the media with the exception of the Ro 20-1724 response ($P = 0.08$) (Table 2). In general, the effect of PDE inhibitors on tissue prorenin content was approximately 10% of media values.

Effect of the combination of type III and IV selective PDE inhibitors with IBMX on prorenin secretion. To demonstrate the relative contribution of type III and IV PDEs in mediating prorenin secretion, selective inhibitors of type III (cilostamide) and IV (Ro 20-1724) along with zardaverine (types III and IV) were used in combination with the non-selective inhibitor, IBMX (Fig. 2). The combination of submaximal stimulatory concentrations of cilo-

Table 2. Effect of phosphodiesterase (PDE) inhibitors on placental explant tissue prorenin content

PDE inhibitor	Primary target (PDE)	Concentration (μM)	Tissue prorenin content (μU/mg protein)
Control			1.2 ± 0.1
IBMX	Non-selective	5	1.3 ± 0.0
		50	7.9 ± 0.8*
		500	18.4 ± 2.1*
Cilostamide	Type III	1	1.1 ± 0.2
	cGMP-inhibited	10	4.4 ± 0.2*
		100	7.8 ± 0.7*
Ro 20-1724	Type IV	50	1.2 ± 0.1
	cAMP-specific	500	2.0 ± 0.3†
Zardaverine	Types III and IV	10	1.1 ± 0.0
		100	3.4 ± 0.3*
		1000	6.8 ± 0.7*
8-MeoM-IBMX	Type I	10	1.2 ± 0.1
	Ca^{2+}/calmodulin-dependent	100	1.0 ± 0.0
Dipyridamole	Type V	4	1.2 ± 0.0
	cGMP-specific	40	1.1 ± 0.1
Zaprinast	Type V	5	0.9 ± 0.1
	cGMP-specific	50	1.1 ± 0.1
		500	1.3 ± 0.1

Values are means ± SEM; N = 4 placentas, each with 6 replicates.
*† Significantly different from control; * $P < 0.05$, and † $P = 0.08$.

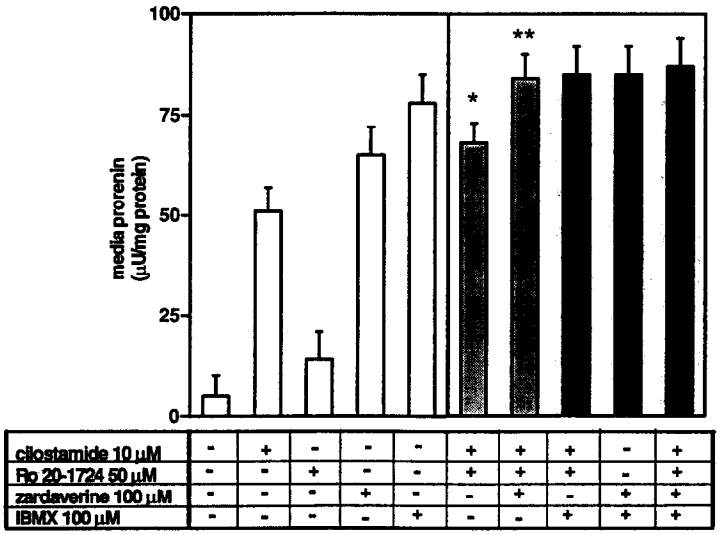

Fig. 2. Effect of the combination of type III and IV PDE inhibitors with the non-selective inhibitor on placental prorenin secretion. Concentrations of prorenin in the medium were determined after 48 hr of incubation. Values represent the mean ± SEM prorenin concentrations in media from 3 placentas with 6 replicates of each. Left panel shows the effects of incubation with a single PDE inhibitor (open bars): Column 1 = control; 2 = type III PDE inhibitor, cilostamide; 3 = type IV PDE inhibitor, Ro 20-1724; 4 = type III and IC PDE inhibitor, zardaverine; 5 = non-selective PDE inhibitor, isobutylmethylxanthine (IBMX). Right panel shows the effects of incubation with combinations of selective PDE inhibitors (shaded bars) and with IBMX (solid bars): 6 = cilostamide + Ro 20-1724; 7 = cilostamide + Ro 20-1724 + zardaverine; 8 = cilostamide + Ro 20-1724 + IBMX; 9 = zardaverine + IBMX; and 10 = cilostamide + Ro 20-1724 + zardaverine + IBMX. Key: (*) different from lane 2, $P < 0.05$; and (**) different from lane 6, $P < 0.05$.

Table 3. Effect of phosphodiesterase (PDE) inhibitors on chorionic gonadotropin secretion by human placental explants

PDE inhibitor	Primary target (PDE)	Concentration (μM)	Medium hCG concentration (mIU/mg protein)	
			−Terbutaline	+Terbutaline (100 μM)
Control			123 ± 10	748 ± 24
IBMX	Non-selective	5	127 ± 12	823 ± 53*
		50	125 ± 8	1150 ± 84*
		500	129 ± 13	1420 ± 75*
Cilostamide	Type III	1	128 ± 10	880 ± 60*
	cGMP-inhibited	10	124 ± 8	1110 ± 82*
Ro 20-1724	Type IV	50	124 ± 7	820 ± 43*
	cAMP-specific	500	127 ± 10	990 ± 50*
Zardaverine	Types III and IV	10	126 ± 6	765 ± 50
		100	124 ± 8	930 ± 70*
		1000	129 ± 7	1320 ± 110*
8-MeoM-IBMX	Type I	100	126 ± 6	754 ± 40
	Ca^{2+}/calmodulin-dependent			
Dipyridamole	Type V	4	121 ± 7	752 ± 30
	cGMP-specific	40	124 ± 8	749 ± 42
Zaprinast	Type V	50	122 ± 6	755 ± 28
	cGMP-specific	500	124 ± 7	751 ± 33

Values are means ± SEM; N = 4 placentas, each with 6 replicates.
* Significant potentiation, $P < 0.05$.

stamide and Ro 20-1724 resulted in an additive response (68.3 ± 4.6 μU/mg protein) which approximated that seen with 100 μM zardaverine (65.1 ± 5.1 μU/mg protein), but was significantly less than that observed with 100 μM IBMX (82.3 ± 7.8 μU/mg protein, $P < 0.05$). The combination of cilostamide, Ro 20-1724 and zardaverine resulted in a greater prorenin response than observed with cilostamide and Ro 20-1724. Co-incubation of zardaverine with the non-selective inhibitor, IBMX, resulted in nearly a 30% increase in prorenin secretion observed with zardaverine alone. Furthermore, the addition of all three selective inhibitors with IBMX yielded no further enhancement of prorenin secretion.

Concentration–response effects of phosphodiesterase inhibitors on chorionic gonadotropin (hCG) secretion by human placental explants. Similar to placental prorenin, the secretion of chorionic gonadotropin is stimulated by β-adrenoceptor agonists and potentiated by selective PDE inhibition [13]. The effects of PDE inhibitors on the secretion of hCG are shown in Table 3. No significant stimulation of hCG was observed with either non-selective or selective inhibition of PDE. However, the response elicited by terbutaline, a β_2-adrenoceptor agonist, was potentiated by PDE inhibitors. Maximal potentiation of 60% was observed with non-selective PDE inhibition by IBMX, and the selective inhibitors cilostamide, Ro 20-1724 and zardaverine each resulted in significant potentiation. Type I and V PDE inhibitors produced no potentiation of the terbutaline response.

Relationship between media cAMP concentration and prorenin secretion by human placental explants treated with PDE inhibitors. To determine if prorenin secretion induced by PDE inhibitors could be attributed to altered cyclic nucleotide metabolism, medium concentrations of cAMP were measured. Placental explants were incubated for 48 hr with a concentration of PDE inhibitor that provided maximal stimulation of prorenin. The medium concentrations of cAMP in the control samples were below detectable levels. Medium cAMP concentrations after 48 hr of incubation with Ro 20-1724 (250 μM), cilostamide (0.5 μM) and IBMX (500 μM) were 3.8 ± 0.1, 4.3 ± 0.2 and 8.0 ± 0.5 pmol/mg protein, respectively (Fig. 3). The combination of cilostamide and Ro 20-1724 increased medium cAMP values to 7.8 ± 0.2 pmol/mg protein. In general, medium cAMP values paralleled prorenin values.

Effects of cAMP-dependent protein kinase inhibitors on prorenin secretion induced by PDE inhibition. A selective inhibitor of cAMP-dependent protein kinase was used to determine if the stimulatory effect of PDE inhibitors on placental explant prorenin secretion was mediated by cAMP. In these studies, the maximal stimulatory concentrations of cilostamide, Ro 20-1724, zardaverine and IBMX were used in combination with a highly selective cAMP-dependent protein kinase inhibitor, H-89. The concentrations of H-89 used in these experiments had no effect on basal secretion of prorenin. Prorenin secretion evoked by the PDE inhibitors IBMX, cilostamide and Ro 20-1724 was inhibited by H-89 in a concentration-dependent fashion (Fig. 4). At 30 μM H-89, PDE inhibitor-stimulated prorenin secretion was reduced by over 60% from the values observed with each selective PDE inhibitor or the combination cilostamide and Ro 20-1724.

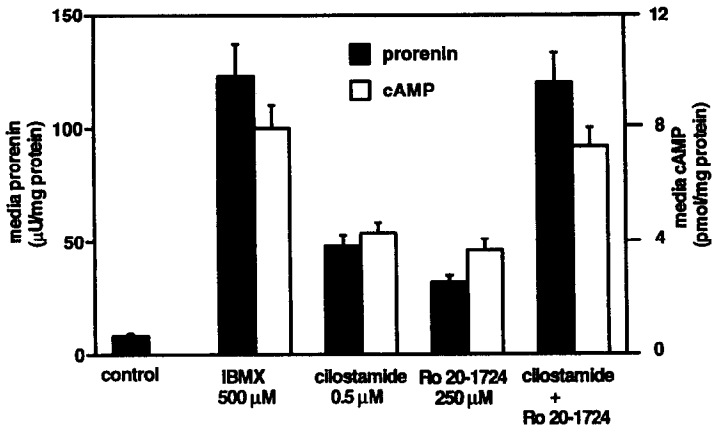

Fig. 3. Relationship between prorenin and cAMP release by placental explants in response to PDE inhibition. Values represent the mean ± SEM concentrations of prorenin and cAMP in medium after 48 hr of incubation. Samples were obtained from 3 separate experiments with a minimum of 3 replicates.

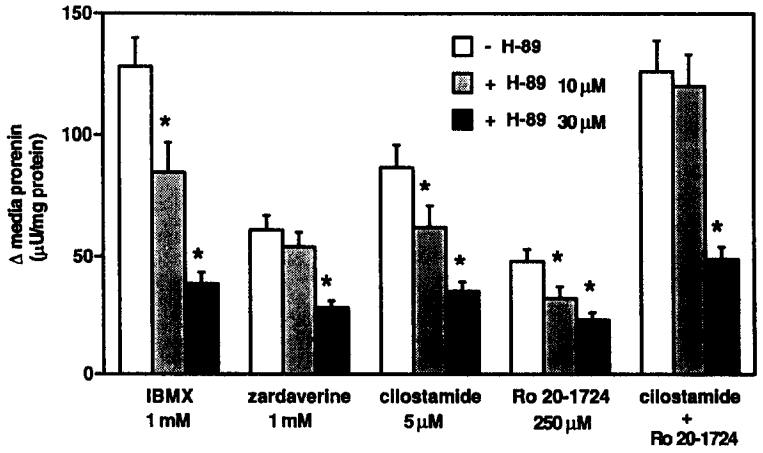

Fig. 4. Effects of cAMP-dependent protein kinase inhibitor, H-89, on PDE-induced stimulation of prorenin secretion by placental explants. Concentrations of prorenin in the medium were determined after 48 hr of incubation. Explants were treated with control media (open bars), H-89 10 μM (shaded bars) or H-89 30 μM (solid bars) for 2 hr prior to treatment with the representative PDE inhibitor(s). Values represent the mean ± SEM for a change in concentrations of prorenin in the medium relative to controls (5.3 ± 0.4 μU/mg protein). Medium concentrations of prorenin after 48 hr of incubation with 10 and 30 μM H-89 were 5.2 ± 0.4 and 4.9 ± 0.5 μU/mg protein, respectively. Key: (*) significant difference from treatment with PDE inhibitor alone, $P < 0.05$. Samples were obtained from 4 separate experiments with a minimum of 3 replicates.

DISCUSSION

Several lines of evidence exist which establish a basis for investigating the role of PDE in placental prorenin regulation. Adenylate cyclase, cAMP and cAMP-dependent protein kinase have been localized in placental tissue and implicated in the regulation of hormone secretion [24]. Multiple PDE isoforms have been localized to the plasma membrane of placental cells and their activity has been characterized [24–27]. Additionally, prorenin secretion from villous placenta is stimulated following activation of adenylate cyclase and non-selective PDE inhibition potentiates this response, thus implicating cAMP as a mediator of prorenin secretion [13]. Therefore, we sought to explore the role of specific subtypes of PDE controlling the synthesis and secretion of prorenin in placenta.

These studies were conducted with an *in vitro* system that permits the study of secretory events from placental tissue [22]. Concentration–response relationships of PDE inhibition on prorenin secretion by human placental explants were evaluated after 48 hr of incubation. Earlier studies had demonstrated

that the maximal stimulation of secretion by β-adrenoceptor agonists occurred during the second 24-hr interval of incubation; therefore, this period was used for these studies [13, 28].

Others have suggested that non-selective PDE inhibitors such as IBMX and theophylline may influence placental secretion by effects on adenosine receptors and intracellular calcium stores [29, 30]. To avoid the possibility of non-cAMP-mediated responses on prorenin secretion, highly selective inhibitors for each PDE family were used [14].

The non-selective PDE inhibitor IBMX, as well as selective inhibitors of type III (cilostamide) and IV (Ro 20-1724) PDEs stimulated prorenin secretion in a concentration-dependent fashion. Inhibitors of type I (8-MeoM-IBMX) and V (dipyridamole or zaprinast) PDE isoforms had no effect. Earlier studies that evaluated the potentiation of β-adrenoceptor-evoked prorenin secretion yielded significant responses with the same PDE inhibitors [13]. In addition, we measured the tissue content of prorenin after 48 hr of incubation. The amount released in the medium and the tissue concentrations after incubation exceeded that found in the controls and freshly homogenized tissue. Taken together, these findings indicate that PDE inhibitors stimulate prorenin synthesis and, subsequently, enhance prorenin release. These effects of the selective inhibitors of PDE are not surprising in view of the relative abundance of type III and IV isoforms in placental tissue and their role in cAMP degradation.

To evaluate the relative contribution of type III and IV PDE isoforms in mediating prorenin secretion, combinations of selective and non-selective inhibitors were studied. Previous reports have indicated that cilostamide and Ro-20 1724 are highly selective for type III and IV PDEs, respectively [31]. Inhibition of type III PDE by cilostamide resulted in prorenin secretion that exceeded that of type IV PDE by Ro 20-1724, thus indicating a greater role of the type III PDE in regulating cAMP-mediated prorenin secretion. The combination of cilostamide and Ro 20-1724 resulted in an additive response as predicted by selectively inhibiting both isoforms, thus arguing against the loss of specificity in the effects of the inhibitors. This response was similar in magnitude to that observed with relatively high concentrations of the selective inhibitor of type III and IV PDEs, zardaverine. The co-incubation of zardaverine with the selective inhibitors cilostamide and Ro 20-1724 resulted in further enhancement of prorenin secretion, providing further evidence that inhibition of both isoforms contributes to prorenin secretion. Furthermore, the finding that IBMX did not induce additional prorenin secretion indicates that inhibition of type III and IV PDEs accounts for effects of this non-specific inhibitor.

The results with PDE inhibitors parallel earlier findings with β-adrenoceptor-mediated prorenin secretion [13] but contrast with studies on decidual explants in which PDE inhibition did not enhance prorenin secretion [28]. The findings are also in contrast to kidney tissue culture studies in which PDE inhibition did not stimulate prorenin secretion independent of adenylate cyclase activation [32].

Moreover, stimulation of prorenin secretion by PDE inhibitors as demonstrated in this system is consistent with active cAMP formation under basal conditions. The fact that PDE inhibition in the absence of exogenous secretagogues induces prorenin secretion suggests that adenylate cyclase is constitutively active in placental explants or that endogenous activators of adenylate cyclase, such as prostaglandins [33], are released during incubation.

In contrast to effects on prorenin, no enhancement of hCG secretion was observed with any of the PDE inhibitors. The responsiveness of the explants to these agents was tested further in combination with terbutaline, a β_2-adrenergic agonist. Significant potentiation of terbutaline-induced hCG secretion was observed with non-selective and type III and IV PDE selective inhibitors. These findings establish differences between the glycoprotein hormone, hCG, and prorenin secretory responses to PDE inhibition.

Medium concentrations of cAMP were enhanced by PDE inhibitors in parallel with medium prorenin values. Although cAMP-dependent protein kinase activity was not assessed in this study, these data support the view that the effects of PDE inhibitors on placental prorenin secretion are mediated by decreased cAMP metabolism and mitigate against the suggestion that PDE inhibitor effects are mediated directly by activation of cAMP-dependent protein kinase [34]. This is consistent with the observations that β-adrenoceptor-mediated prorenin secretion from placenta is associated with increased cAMP release and enhanced cAMP-dependent protein kinase activity [35]. While selective inhibition of cGMP-specific PDE did not influence placental prorenin secretion, this isoform may assume important regulatory features when guanylate cyclase is activated, as evidenced by the effects of nitric oxide on renal renin secretion [36].

Placental prorenin secretion induced by selective and non-selective PDE inhibition was attenuated by an inhibitor of cAMP-dependent protein kinase, H-89, in a concentration-dependent fashion. Although these results suggest that PDE inhibitor-evoked prorenin secretion is mediated by cAMP-dependent kinase, cAMP effects independent of cAMP-dependent kinase activation cannot be excluded completely. Additionally, direct cAMP activation of voltage-gated ion channels may be operative, and additional kinases may influence prorenin secretion via cAMP signaling.

In conclusion, these results demonstrate that PDE metabolism of cAMP is a regulatory step in placental prorenin synthesis and secretion. The PDE inhibition-associated prorenin secretion in the absence of exogenous secretagogues suggests the presence of paracrine/autocrine activators of adenylate cyclase.

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