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DIFFERENTIAL ROLE OF MICROTUBULES IN THE CONTROL OF PROSTAGLANDIN E_2 AND β-ADRENERGIC STIMULATION OF CYCLIC AMP ACCUMULATION IN THE RAT MYOMETRIUM

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Abstract—A possible modulatory role of microtubules was investigated for the β-adrenergic and prostaglandin E_2 (PGE_2)-induced cyclic AMP accumulation in the estrogen-treated rat myometrium. Colchicine, vinblastine and nocodazole, three different antitubulin drugs, enhanced cyclic AMP accumulation caused by PGE_2. The effect of inhibitors was dose-(0.1–5 μM) and time-dependent, increased maximal responses without changing EC_{50} for PGE_2, did not occur with trimethyl-colchicinic acid, which does not bind to tubulin, and was totally prevented in tissues pretreated with taxol, an agent that enhances polymerization and stabilization of microtubules. Concomitantly, colchicine reduced the rate and extent of PGE_2-induced refractoriness in terms of cyclic AMP. In contrast, the antitubulin drugs failed to affect the rise in cyclic AMP evoked by isoproterenol and cholera toxin but enhanced the response to prostacyclin (PGI_2), which is assumed to share common receptors with PGE_2. Colchicine and vinblastine also failed to alter the ability of the β-adrenergic agonist to provoke a cyclic AMP refractory state. Stimulations induced by all effectors were totally insensitive to cytochalasin B. The data suggest a relation between the constraints associated with the microtubules and/or membrane tubulin of the myometrium and the efficacy of PGE_2 and PGI_2 (but not the β-adrenergic agonist or cholera toxin) to interact with the cyclic AMP forming system.

In previous work from this laboratory, it has been shown that the estrogen-dominated rat myometrium has the capacity to synthesize cyclic AMP in response to a β-adrenergic agonist as well as to PGE_2† (and to prostacyclin 'PGI_2'). These activations involve distinct receptors for both catecholamines and prostaglandins [1, 2]. It is well documented that hormone-regulated cyclic AMP formation, occurring via the adenylate cyclase system, is the result of an interaction of at least three membrane-associated components: the hormone receptor, the regulatory or guanyl nucleotide binding unit (N-unit) and the catalytic moiety of the cyclase [3]. Many cell surface receptors and ligand binding sites appear to be mobile in the plane of the plasma membrane and this receptor mobility may be regulated by various factors intrinsic to, or associated with, the plasma membrane [4–6]. The possibility that microtubules might regulate the mobility of receptor proteins within the membrane and hence interfere with receptor–N-unit or –cyclase interactions has recently been addressed. Several reports have indeed indicated that colchicine, a microtubule disrupting drug, augments β-adrenergic- (and hormone-) stimulated cyclic AMP synthesis in different cell preparations [7–10] and may modulate in some [11], but not all [12], cells β-adrenergic receptor binding properties. More recently, an interaction of the regulatory unit of the adenylate cyclase system with cytoskeleton components has also been reported [13, 14].

The present study attempts to clarify whether microtubules and/or membrane tubulin exert some regulatory control on the response of the intact myometrium to two different effectors, viz. β-adrenergic and PGE_2, in relation to the efficacy of cyclic AMP formation and the ability of the tissue to terminate the stimulus response and to develop desensitization [15]. As an approach to such an investigation, colchicine and vinblastine, two inhibitors of microtubule assembly and presumably of microtubule (or tubulin) function [16, 17], were employed. The data demonstrate that the antitubulin drugs enhanced cyclic AMP stimulation and inhibited cyclic AMP refractoriness induced by PGE_2 while they did not alter isoproterenol responses either at the stimulation or at the desensitization level, implying differential interaction of the tubulin system with the β-adrenergic- and PGE_2-(also PGI_2-) linked adenylate cyclase activation in the intact myometrium.

MATERIALS AND METHODS

Chemicals. Isoproterenol bitartrate was obtained from Fluka A.G. (Switzerland); colchicine, trimethyl-colchicinic acid, vinblastine sulfate, cytochalasin B, cholera toxin '*Vibrio cholerae*' β-oestradiol-3-benzoate were from Sigma Chemical Co. (St Louis, MO); nocodazole was from Calbiochem (San Diego, CA); cyclic AMP was from P.L. Biochemical (Cleveland, OH); 3-isobutyl-l-methylxanthine was from Aldrich Chemical Co. Inc. (Milwaukee, WI); [^{3}H]cyclic AMP (~34 Ci/mmole) was from New England Nuclear Inc. (Dreieich, F.R.G.); and PGI_2 and PGE_2 were gifts from the

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† Abbreviations: PGE_2, prostaglandin E_2; PGI_2, prostacyclin; MIX, 3-isobutyl-1-methylxanthine.

Upjohn Co. (Kalamazoo, U.S.A.). Indomethacin was kindly supplied by Merck, Sharp & Dohme (Rahway, NJ). Taxol was a gift to Dr. L. Rappaport from Dr G. B. Douros, Chief of Natural Product Branch, Division of Cancer Treatment, NIH, Bethesda, U.S.A.

Animals and tissue processing. Uteri were obtained from estrogen-pretreated rats (Wistar, 4–5 weeks old), and myometrium was prepared free of endometrium as previously described [1, 15].

Incubation experiments for assay of cyclic AMP accumulation. Incubations were carried out in Krebs–Ringer bicarbonate buffer at 37° (gas phase 95% O_2, 5% CO_2) under constant agitation. Myometrial strips (about 50 mg) were added to 2.0 ml of buffer and allowed to equilibrate for 25 min at 37°, followed by a 30 min incubation with or without 3 μM colchicine, or with the other drugs when tested. After the addition of 3-isobutyl-1-methylxanthine (MIX), final concentration 57 μM, incubations were further continued for different periods, as described in the figure legends, in the presence of the various agonists to be tested (PGE_2, PGI_2, isoproterenol, cholera toxin) at the indicated concentrations. The reactions were stopped by immersing the tissue strips in 2 ml of cold 7% TCA, followed by homogenization of the samples and centrifugation for 20 min at 30,000 *g*. Cyclic AMP was estimated in the TCA-soluble extracts according to Gilman [18] as previously reported [1, 15]. The centrifuged pellets were dissolved in 2 ml of 1 N NaOH for protein determination [19]. Cyclic AMP levels are expressed as pmole/mg protein.

Desensitization treatment. Following the 25 min equilibration period and the preincubation step with or without colchicine, myometrial strips were initially exposed to either 10 μM isoproterenol or 30 μM PGE_2 at 37° for various times, depending on the experiment [15]. The tissue strips were subsequently washed three times with 10 ml of hormone-free buffer, then transferred to 2.0 ml of fresh buffer and allowed to equilibrate 5 min at 37°. Rechallenge experiments were routinely conducted for 10 min in the presence of 57 μM MIX and the indicated agonist (0.1 μM isoproterenol or 10 μM PGE_2) to be tested on the pretreated tissue. Cyclic AMP was then extracted and assayed as described above.

Statistical analysis. The results are expressed as mean ± S.E. and were analysed statistically using student's *t*-test. P values of <0.05 were accepted as significant.

RESULTS

Effects of colchicine on PGE_2- and isoproterenol-induced cyclic AMP accumulation in the rat myometrium

As reported previously [15] and shown in Fig. 1, PGE_2 and isoproterenol, at their maximal effective concentrations, and in the presence of 57 μM MIX, enhanced a 8 to 10-fold increase in intracellular cyclic AMP content of the rat myometrium. PGE_2 and isoproterenol stimulatory effects were rapidly detectable, reaching within 2–3 min a plateau value which could be sustained up to 20 min incubation with both agonists. When myometrial strips were preincubated with 3 μM colchicine for 1 hr at 37°, prior to the addition of PGE_2, there was a 2-fold increase in the PGE_2 response in terms of cyclic AMP accumulation. The effect of colchicine was equally manifested at the different incubation times tested (30 sec–15 min). By contrast, no alteration in the cyclic AMP stimulations caused by isoproterenol could be detected after the colchicine treatment. Similarly, colchicine, when added alone, was unable to modify basal cyclic AMP levels significantly (5.4×0.6 and 4.9 ± 0.6 pmole cyclic AMP/mg protein in control and colchicine-treated tissue, respectively) as well as cyclic AMP levels reached in the presence of MIX (8.2 ± 0.7 and 7.9 ± 0.8 pmole cyclic AMP/mg protein in control and treated tissue, respectively).

The potentiating effect of colchicine on PGE_2-

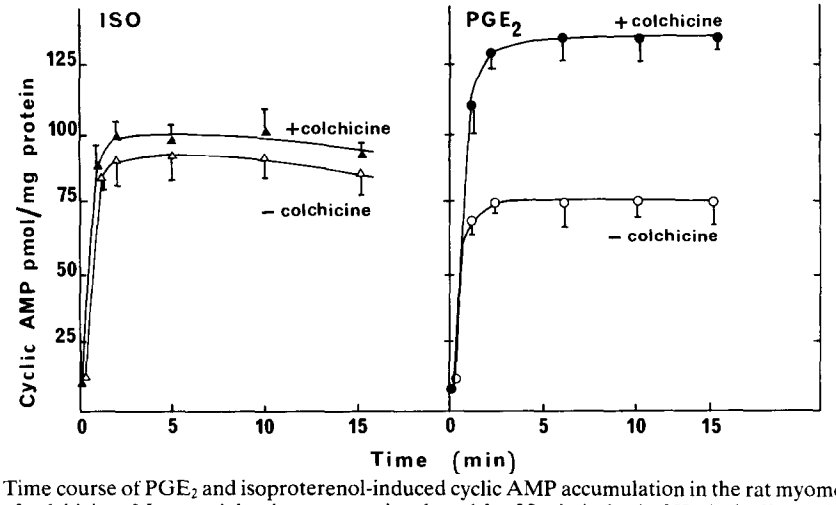

Fig. 1. Time course of PGE_2 and isoproterenol-induced cyclic AMP accumulation in the rat myometrium. Effect of colchicine. Myometrial strips were preincubated for 25 min in 2 ml of Krebs buffer and further incubated for 30 min in the absence (open symbols) and presence (closed symbols) of 3 μM colchicine. MIX (57 μM) was then added, tissues were incubated for 5 min and challenged with 0.1 μM isoproterenol (△, ▲) on 10 μM PGE_2 (○, ●). Reactions were stopped at the indicated times and cyclic AMP was assayed as described in Materials and Methods. Values represent the mean ± S.E. of at least four different experiments.

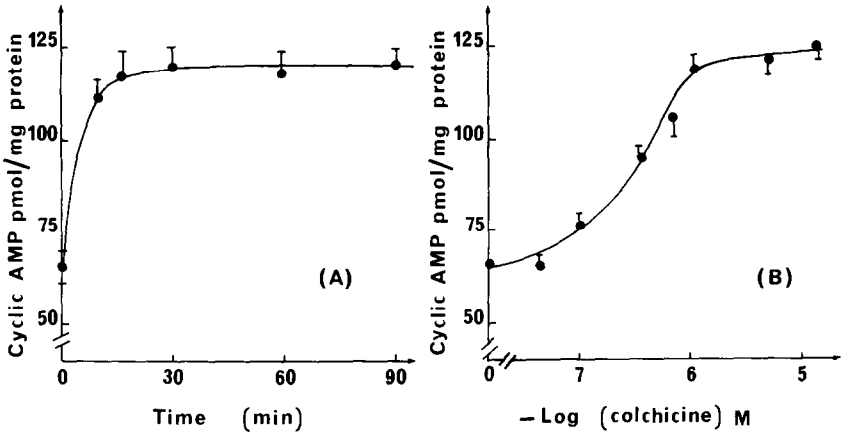

Fig. 2. Kinetics and concentration-dependent effects of colchicine on the rise in cyclic AMP caused by PGE_2. Myometrial strips were treated with 3 μM colchicine for the indicated times (a) or with different concentrations of colchicine for 30 min (b) before further incubation with MIX (57 μM) plus PGE_2 (10 μM), and the assay of cyclic AMP, as in legend of Fig. 1. Values represent the mean ± S.E. of three different experiments. In the absence of colchicine, values averaged 7.2 ± 0.8 and 65.7 ± 10 pmole cyclic AMP/mg protein for control and PGE_2-stimulated tissues, respectively.

induced cyclic AMP elevation was dose-dependent (Fig. 2b), being evidenced over a narrow range of concentrations, with a slight stimulation detected at 0.1 μM and a virtually maximal effect at 1 μM colchicine. Again, increasing drug concentration up to 100 μM did not provoke any enhancement in the cyclic AMP response due to the β-adrenergic agonist. Preincubation with colchicine showed a time-dependence for drug-mediated increase in PGE_2 response on cyclic AMP, with a 20–30 min period required for maximal enhancement by 3 μM colchicine (Fig. 2a). In subsequent studies, myometrial strips were routinely incubated with 3 μM colchicine for at least 30 min at 37°.

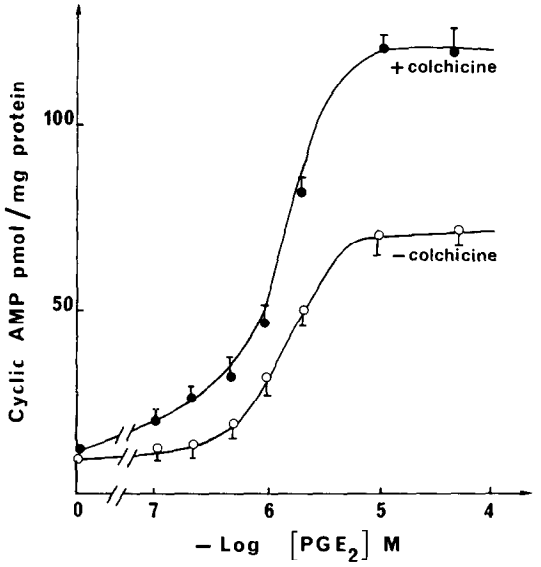

Fig. 3. Effect of colchicine on cyclic AMP levels induced by different concentrations of PGE_2. Myometrial strips were preincubated for 30 min in the absence (○) or presence (●) of 3 μM colchicine. MIX was then added to a final concentration of 57 μM; incubation continued for 5 min before the addition of PGE_2 at the indicated final concentrations. After 10 min further incubation, cyclic AMP was determined as described in Materials and Methods. Values represent the mean ± S.E. of three to five different experiments each carried out in duplicate.

Dose–response curves for PGE_2-induced cyclic AMP accumulation were determined in the absence and presence of 3 μM colchicine (Fig. 3). Over the whole range of PGE_2 concentrations tested, colchicine enhanced the PGE_2 responses, with an approximate 2-fold increase in the maximal PGE_2 stimulations. However, colchicine did not affect the EC_{50} for PGE_2 (3 μM for the control and 4 μM for the colchicine-treated myometrium). Colchicine was similarly without effect on cyclic AMP stimulations caused by supramaximal as well as submaximal concentrations of the β-adrenergic agonist (without colchicine, 50.5 ± 7, 79.8 ± 12 and 87.8 + 11; and with colchicine, 51.6 ± 7, 74.4 ± 14 and 82.7 ± 14 pmole cyclic AMP/mg protein at 0.02, 0.05 and 0.1 μM isoproterenol, respectively).

Vinblastine (1 μM) and nocodazole (10 μM), two structurally different drugs which bind to tubulin and disrupt microtubules [17, 20], showed the same effect as colchicine, with a 2-fold increase in PGE_2-induced cyclic AMP accumulation and still no effect on isoproterenol stimulations. Trimethyl-colchicinic acid, which does not bind to tubulin [21], did not enhance PGE_2- or isoproterenol-induced cyclic AMP accumulation (Table 1). No effect of trimethyl-colchicinic acid on the response to either agonist could be observed even when the drug concentration was raised to 10 μM with the preincubation time ranging from 15 to 60 min (results not shown). Cytochalasin B, an agent known to interfere with microfilament structures, had virtually no effect on PGE_2 stimulations. When cytochalasin B was combined with colchicine, the apparent effect found was that of colchicine alone. Cytochalasin B also did not modify the isoproterenol response in terms of cyclic AMP (Table 1). Incubation of the myometrium with 2 μM taxol, which promotes and stabilizes microtubule assembly [24, 25], prior to the addition of colchicine,

Table 1. Effect of various drugs on cyclic AMP accumulation induced by PGE_2 and isoproterenol

Pretreatment	Control	PGE_2 (pmole cyclic AMP/mg protein)	Isoproterenol
None	8.1 ± 0.7	70.3 ± 8.5	92.8 ± 10.6
Colchicine, 3 μM	7.8 ± 0.8	113.0 ± 9.7	98.6 ± 7.5
Vinblastine, 1 μM	7.6 ± 0.9	138.8 ± 23	98.4 ± 10
Nocodazole, 10 μM	8.4 ± 1.1	112.9 ± 7.0	90.7 ± 10
Trimethyl-colchicinic acid, 3 μM	7.8 ± 1.0	65.7 ± 7.0	89.8 ± 9
Cytochalasin B, 10 μM	6.9 ± 1.5	70.8 ± 9.5	89.2 ± 10
Colchicine, 3 μM + cytochalasin B, 10 μM		116.9 ± 7.5	90.4 ± 13

Myometrial strips were preincubated for 30 min at 37° in the presence of the indicated drugs. Incubation was then carried out in the presence of 57 μM MIX for 5 min and further continued for an additional 10 min in the absence (control) or presence of 0.1 μM isoproterenol and 10 μM PGE_2. Cyclic AMP was estimated as described in Materials and Methods. Values are the mean ±S.E. of three to five experiments each carried out in duplicate.

resulted in a complete loss of the potentiating effect of colchicine on PGE_2 responses in terms of cyclic AMP (Table 2). Under these conditions, taxol did not affect basal cyclic AMP levels or the stimulations normally caused by PGE_2 in the absence of the antitubulin drug. By contrast, when the myometrium was initially preincubated with colchicine prior to the subsequent addition of taxol, the potentiating effect of colchicine on PGE_2-mediated cyclic AMP response was maintained. This finding is consistent with a recent observation that under non-polymerizing conditions [^{3}H]taxol does not bind with high affinity to the tubulin dimer [26].

Experiments were then performed under comparable conditions to examine the response of the myometrium to other adenylate cyclase stimulating agents, viz. prostacyclin 'PGI_2', which is a potent activator of cyclic AMP accumulation in the myometrium and which has been postulated to share common receptors to PGE_2 [2, 15], and cholera toxin [27]. The results in Table 3 demonstrate that cholera toxin induced cyclic AMP accumulation in the rat myometrium (maximal effect was reached at 30 nM of the toxin, after an incubation time of 1 hr, not shown). Colchicine did not modify the cyclic AMP response to cholera toxin but enhanced PGI_2-evoked cyclic AMP elevation. In addition, the results of Table 3 show that the enhancement PGE_2 response caused by colchicine could not be ascribed to a local increase in the generation of prostaglandin material(s) inasmuch as stimulations were sill evidenced in the presence of indomethacin, under conditions where endogenous synthesis of prostaglandins by the myometrium is totally abolished [2]. Isoproterenol stimulations were similarly unaffected by indomethacin. Taken as a whole, the data favour the interpretation that colchicine-pretreatment does not seem to affect some site that is common to the action of different hormones and cholera toxin on the cyclic AMP forming system, but rather a component involved in the PGE_2- (and PGI_2-) interaction with its putative receptor and/or coupling with the adenylate cyclase catalytic unit.

The effect of colchicine on isoproterenol- and PGE_2-induced cyclic AMP refractoriness

It has been demonstrated that *in vitro* exposure of the rat myometrium to either isoproterenol or PGE_2 results in a subsequent refractory state of the tissue cyclic AMP response [15]. In order to deter-

Table 2. Effect of taxol on the enhancement by colchicine of PGE_2-mediated cyclic AMP accumulation

First treatment	Second treatment	Basal (pmole cyclic AMP/mg protein)	PGE_2
None	None	11.8 ± 1.4	77.6 ± 10.4
Colchicine, 3 μM	None	12.2 ± 1.8	160.6 ± 23.6
Taxol, 2 μM	None	10.7 ± 1.4	76.4 ± 1.4
Taxol, 2 μM	Colchicine, 6 μM	n.d.*	87.2 ± 13
Colchicine, 3 μM	Taxol, 2 μM	n.d.	170.2 ± 13.5

Myometrial strips were submitted to either a single pretreatment with or without 3 μM colchicine or 2 μM taxol for 30 min, or to two successive treatments: 30 min with 3 μM colchicine followed by 30 min incubation with taxol and vice versa. Tissues were then further incubated in the presence of MIX (57 μM) with or without the addition of 10 μM PGE_2 for cyclic AMP estimations as in the legend of Fig. 1. Values are the mean ± S.E. of three different experiments.

*n.d. = Not determined.

Table 3. Selective enhancement by colchicine of cyclic AMP accumulation induced by PGE_2 and prostacyclin. Effect of indomethacin

Pretreatment	PGE_2, 10 μM	PGI_2, 5 μM	Isoproterenol, 0.1 μM	Choleratoxin, 30 nM
		(pmole cyclic AMP/mg protein)		
None	71.3 ± 7.9	79.6 ± 8	92.8 ± 15.6	77.5 ± 8.3
Colchicine, 3 μM	115.7 ± 9.2	127.6 ± 56	98.6 ± 6.7	77.8 ± 7.0
Indomethacin, 5 μM	70.8 ± 8	n.d.*	91.7 ± 10	n.d.
Indomethacin, 5 μM+ colchicine 3 μM	115.3 ± 6.6	n.d.	90.5 ± 7	n.d.

Myometrial strips were pretreated for 30 min, with 3 μM colchicine and further incubated in the presence of MIX (57 μM) with the addition of PGE_2, PGI_2 and isoproterenol at their respective concentrations for 10 min, or with the addition of 30 nM choleratoxin for 1 hr before the assay of cyclic AMP, essentially as in the legend of Table 1. Indomethacin (5 μM), when used, was added to the incubated tissue 5 min before the colchicine treatment. Values are the mean ± S.E. of three to five different experiments.

* n.d. = Not determined.

mine whether a microtubule disrupting drug exerted any effect at the level of the cyclic AMP refractoriness phenomenon, control and colchicine-treated myometrial strips were exposed to either isoproterenol or PGE_2 for varying periods, then washed to remove the agonist and challenged with a fresh medium containing isoproterenol or PGE_2. The extent of responsiveness to the latter agonists is illustrated in Fig. 4. It is interesting to note that tissues which had been incubated with colchicine or vinblastine and subsequently washed maintained their enhanced response to PGE_2 for as long as 4–6 hr after removal of the drug (data not shown).

In the absence of colchicine, self-induced refractoriness, i.e. PGE_2 vs PGE_2, and isoproterenol vs isoproterenol (Fig. 4 a and b), was rapid, almost complete by 30 min with almost 70% decline in the corresponding agonist cyclic AMP response. Pretreatment of the myometrium with colchicine prior to isoproterenol exposure did not affect the β-adrenergic-induced self refractoriness: both the kinetic pattern and the degree of refractoriness to

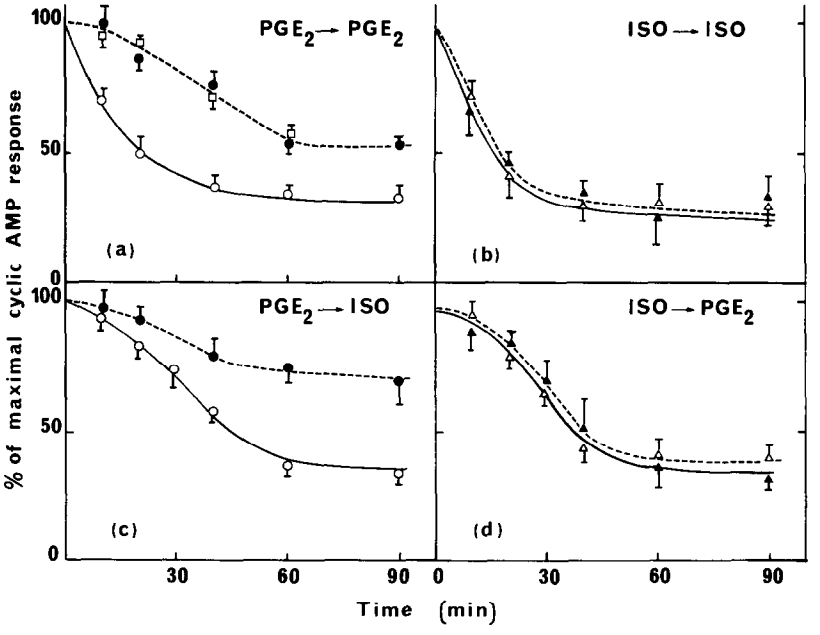

Fig. 4. Effect of colchicine on the development of PGE_2 and isoproterenol-induced specific and cross-desensitization. After a 30 min incubation in the absence (○, △) or presence of 3 μM colchicine (●, ▲) or 1 μM vinblastine (□), myometrial strips were exposed to 30 μM PGE_2 (○, ●, □) and 10 μM isoproterenol (△, ▲) for the times indicated. The agonists were then removed by three successive washings with 10 ml hormone- and colchicine-free buffer. The tissues initially exposed to PGE_2 were then challenged with 10 μM PGE_2 (a) or 0.3 μM isoproterenol (c). Tissues initially exposed to isoproterenol were then challenged with 0.3 μM isoproterenol (b) or 10 μM PGE_2 (d). Rechallenge experiments were carried out in the presence of MIX (57 μM) for 10 min and cyclic AMP was determined as described in Materials and Methods. Results are expressed as the percentage of the response of the control tissue to the corresponding agonist (in the absence or presence of colchicine). For control tissues, cyclic AMP responses were without colchicine 69 ± 7.8 and 92.8 ± 10.6, and with colchicine 115.7 + 9.2 and 98.6 + 10 pmole cyclic AMP/mg protein for PGE_2 and isoproterenol stimulations, respectively.

isoproterenol were virtually identical in the absence or presence of colchicine. By contrast, colchicine markedly affected PGE_2-induced self-refractoriness inasmuch as there was an important lag in the development of desensitization that became only apparent after 30 min exposure to PGE_2, with a marked reduction in the maximal loss of responsiveness (45%). It is also of interest to note that the decline in cyclic AMP response to PGE_2 was similarly inhibited when exposure of the myometrium to PGE_2 was performed with the simultaneous presence of vinblastine (Fig. 4a). The kinetic patterns of PGE_2-induced cross-desensitization to isoproterenol and isoproterenol-induced cross-desensitization to PGE_2 illustrate (Fig. 4 c and d), as previously reported [15], that the process was slower in onset compared to the agonist-induced specific refractoriness. It is evident that the presence of colchicine during the exposure period to isoproterenol did not affect the subsequent decline in the cyclic AMP response to PGE_2. Nevertheless, colchicine markedly inhibited the ability of PGE_2 to provoke the heterologous desensitization vs isoproterenol.

DISCUSSION

The results reported in the present study show that colchicine enhanced PGE_2- and PGI_2-mediated cyclic AMP accumulation in the rat myometrium. Several lines of evidence indicate that the colchicine effect is due to drug-induced perturbation of microtubule and/or membrane tubulin function [17, 22, 23]. First, the use of three structurally different microtubule disrupting drugs, viz. colchicine, vinblastine and nocodazole, elicited the same changes. Second, trimethyl-colchicinic acid, which does not bind to tubulin, had no effect on PGE_2-induced cyclic AMP accumulation. Third, the concentrations of the different drugs that potentiated the prostaglandin effect on cyclic AMP were similar to those reported for inhibition of tubulin polymerization *in vitro*. Finally, taxol, which promotes microtubule assembly and stabilization, completely prevented the potentiation by colchicine of PGE_2-mediated cyclic AMP effects, the latter findings being consistent with the observations that, in taxol-treated cells, microtubules resisted depolymerization by antitubulin drugs [26].

Since the effects were obtained with tissues incubated in the presence of MIX, a phosphodiesterase inhibitor, it seems likely that the enhancement of PGE_2-mediated cyclic AMP accumulation by antitubulin drugs was due to increase in generation rather than inhibition of cyclic AMP degradation. In the same line, our data demonstrate the specificity of the antitubulin drugs for the PG-induced cyclic AMP accumulation. Indeed, both colchicine and vinblastine failed to affect cyclic AMP responses caused by two other effectors, viz. isoproterenol, whose stimulations are well documented to be mediated by β-adrenergic receptor occupancy and a coupling process involving guanine nucleotide regulatory proteins 'N' [3, 28], and cholera toxin, which, bypassing the receptor step, stimulates adenylate cyclase through a direct activation of N. The similarity of the potentiating effects observed with both PGE_2 and PGI_2 is noteworthy in view of our previous observations [15] that these two PGs appear to share common receptors (distinct from the β-adrenergic) that are coupled to the same catalytic unit of the cyclase. It is also pertinent to note that PGI_2 is locally generated in the myometrium and that it is the major PG material modulating intracellular cyclic AMP content [2].

Colchicine and vinblastine have been reported to enhance β-adrenergic- (and/or PGE_2-) induced cyclic AMP responses in a number of inact cells [7–11]. Activation by the antitubulin drugs has been interpreted to be, in some cases, operating at a step distal to the hormone–receptor interaction and most probably involving the N proteins [8, 9]. Further observations with the rat erythrocytes also suggest that N may interact with cytoskeletal components [13]. On the other hand, some recent data [11] favour the interpretation of a direct cytoskeletal regulation at the level of β-adrenergic receptor properties in live human polymorphonuclear leukocytes. Our present findings seem to indicate that in the intact rat myometrium, the colchicine effect is exerted at a site that is not common to all hormone- and choleratoxin-mediated adenylate cyclase activation, thus precluding a direct effect of the drug at the N–C interaction step. The data rather suggest that the state of microtubule assembly is likely to affect an aspect of receptor function that is unique to PGE_2 (and PGI_2). It is worth mentioning that the EC_{50} for PGE_2-mediated cyclic AMP responses is not modified in the presence of microtubule disrupting drugs.

In tentatively considering the underlying mechanisms for the effect of colchicine in enhancing cyclic AMP accumulation specifically caused by PGE_2, it is possible to speculate that disruption of microtubules might result in an increased mobility of PGE_2 (and PGI_2) receptors in the membrane and hence favour an increased accessibility of the agonist to its receptor and/or an increased probability of coupling between occupied receptors and adenylate cyclase. This interpretation could also implicate a heterogeneous population of PG receptors, some of which can diffuse freely in the membrane (involved in basal PGE_2-responses) and some are immobilized (expressed in colchicine-treated cells). The situation would thus be similar to the recently reported observation of a mixed (free and immobilized) population of antibody receptors [29]. The latter hypothesis has also been advanced to explain the potentiating action of colchicine on β-adrenergic cyclic AMP responses in the polymorphonuclear leukocytes [11]. An alternate interpretation of our findings in the myometrium would be that microtubules are required for the desensitization of PGE_2 receptors by the agonist. This would implicate that the amounts of cyclic AMP accumulated by incubating the myometrium with PGE_2 reflect the net result of both stimulation and desensitization of PGE_2 receptors. Conceivably, when microtubule assembly is inhibited, desensitization would be attenuated and the activation process would predominate. Quite consistent with this assumption are our observations that relaxation of the constraints exerted by the microtubules, through the action of various microtubule disrupting drugs, not only facilitated interaction of PGE_2 with the cyclic AMP forming system but also coincidently retarded and decreased the extent of cyclic AMP

refractoriness induced by PGE_2. For the β-adrenergic agonist, no effect of colchicine could similarly be noted at both the cyclic AMP stimulation or the desensitization responses. We recently reported that the attenuated response to isoproterenol, when elicited by the agonist itself, could readily be explained by both a reduction in the number of β-adrenergic binding sites (i.e. a down-regulation of receptors) and an impaired ability of the residual receptors to form a high affinity complex with the agonist, i.e. an uncoupling state [28]. When isoproterenol-induced self-refractoriness was achieved in the presence of colchicine as in Fig. 4b, similar altered receptor properties were noted (data not shown), supporting the view that the integrity of the microtubules is not a prerequisite for the down-regulation and the uncoupling process at the β-adrenergic receptor level.

Clearly, further studies are required to extend our observations at the level of PGE_2 (and PGI_2) receptors and the coupling of these occupied receptors to adenylate cyclase activation and desensitization. This may help to support one or more of the hypothesized mechanisms noted above in explaining how certain alterations in the cell membrane may render the myometrium more susceptible to regulation by PGE_2 and PGI_2 in terms of cyclic AMP accumulation. Finally, the fact that microtubules apparently do not participate in the isoproterenol-induced cyclic AMP response in the myometrium is in contrast to the reported potentiating effect of colchicine and vinblastine on β-adrenergic-mediated cyclic AMP stimulations in a number of cellular systems [7–10]. These discrepancies may be related to relative variations in the localization, quantity or association to membrane elements of microtubules in different cell types [30]. The differential effect of microtubule disrupting drugs for the β-adrenergic and PGE-mediated cyclic AMP stimulations described here in the myometrium may also be indicative of subtle variations in the membrane micro-environments where β-adrenergic and PGE_2 (also PGI_2) receptors could be located.

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