REGULATION OF GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE LEVELS AND CONTRACTILITY IN RAT MYOMETRIUM

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1. Introduction

There is a large body of evidence suggesting a role for cAMP in mediating smooth muscle relaxation [1–3]. Our own investigations with oestrogen pretreated rat myometrium demonstrated that both the relaxing agent epinephrine and the contracting agent PGE_1 promote similar rises in cAMP levels with the expected saturation of intracellular cAMP receptor proteins [4–6]. These observations were not consistent with the assumption of an exclusive role for the adenylate cyclase-cAMP system in mediating uterine relaxation. On the other hand, marked increases in cGMP levels have been obtained in various types of smooth muscles in response to certain stimulants that promote contraction [1–3]. Nevertheless in a number of cases, cellular cGMP content could not be systematically related to the contractile activity of the muscle [7–9]. The present investigation was designed to evaluate modulation of cGMP levels in rat myometrium in response to various contracting and relaxing agents. The effects of phosphodiesterase inhibitors and guanylate cyclase activators were also examined. The regulation of cGMP accumulation in the myometrium and its possible role in uterine contractility are discussed.

Abbreviations: cAMP, adenosine 3',5'-cyclic monophosphate; cGMP, guanosine 3',5'-cyclic monophosphate; MIX, 3-isobutyl-1-methyl xanthine; EGTA, ethanedioxy-bis-(ethylamine) tetraacetic acid; PG, prostaglandins

2. Materials and methods

2.1. *Chemicals*

cAMP, cGMP (PL Biochemical Inc.), theophylline, carbamylcholine hydrochloride (Merck Darmstadt), 1-methyl-3-isobutyl xanthine (Aldrich), oxytocine, 2'-O-succinyl-cGMP (Sigma Chemical Co.). c[^{3}H]AMP (CEA, France). ^{125}I-labelled succinyl-cGMP tyrosine methyl ester was prepared by P. Pradelles and F. Dray (URIA, Institut Pasteur, France). The antibody used for radioimmunoassay of cGMP was from Institut Pasteur Production, France. Prostaglandins E_2 and $F_{2\alpha}$ were from Upjohn Co.

2.2. *Tissue preparation and incubation*

Uteri were obtained from oestrogen pretreated rats and myometrial strips were prepared free of endometrium. Tissue incubation and preparation of trichloracetic acid extracts were essentially as in [5]. The soluble TCA extracts were used for cAMP and cGMP estimations.

2.3. *Assay of cGMP and cAMP*

cGMP was measured according to the radioimmunoassay method developed [10] after the succinylation step proposed [11] in order to increase the assay sensitivity. Free antigen was separated from the bound antigen–antibody complex by precipitation with cold ethanol (P. Pradelles and F. Dray, personal communication). In each assay series, the extent of succinylation was routinely controlled and averaged a value of 80–90%. In some experiments, prior to cGMP assay, extracts were purified on Dowex AG 1-X

formate columns [12] which adequately separate cAMP from cGMP. The purification step was not routinely conducted, since values obtained were similar to those determined without purification.

cAMP was estimated as described [5] according to [13].

3. Results and discussion

3.1. *Effect of phosphodiesterase inhibitors on cAMP and cGMP content of rat myometrium*

Basal levels of cGMP in rat myometrium equilibrated in Krebs Ringer at 37°C average 0.37 ± 0.04 pmol/mg protein. These conditions yield a cAMP/cGMP ratio equal to 13–15. Two phosphodiesterase inhibitors, theophylline and 1-methyl-3-isobutyl xanthine (MIX) evoked a significant rise in both cGMP and cAMP, in a dose-dependent manner (table 1). However in all cases, the increase in cGMP was more pronounced than that of cAMP. The value for cGMP may reach 10-fold that of the control while that of cAMP under the same conditions was increased by 2-fold. All the tested concentrations of MIX that promoted such increases in cGMP levels exerted also a relaxing effect on rat myometrium and antagonized contractions elicited by prostaglandins (E and F), oxytocin and carbamylcholine.

3.2. *Effect of carbamylcholine and other contracting agents*

It has been shown that cholinergic stimulation in a variety of tissues including different smooth muscle preparations, leads to cellular accumulation of cGMP [3,10,14–16]. Carbamylcholine had a very slight stimulatory effect on cGMP accumulation in rat myometrium (0.44 ± 0.05 and 0.53 ± 0.05 pmol cGMP/mg protein with 50 μM and 100 μM carbamylcholine, respectively). It was however difficult to conclude to any consistent stimulatory effect since in the presence of either phosphodiesterase inhibitor, theophylline or MIX, cGMP levels were not modified by the addition of different concentrations of carbamylcholine. The ability of the cholinergic agent to induce a rise in cGMP could not be improved by increasing its concentration up to 500 μM, whether tension was applied or not on uterine segments and whether cGMP was measured in the tissue alone or in the tissue plus incubation medium. Under such conditions, carbamylcholine evoked myometrial contractions, and this effec was prevented by atropine. Hence the present findings confirm some previous observations [7,17] and suggest that the rat uterus appears to be an exception where stimulation of muscarinic receptors does not lead to consistent increases in tissue cGMP content.

Moreover, a series of other contracting agents,

Table 1
Effect of phosphodiesterase inhibitors on cGMP and cAMP contents of rat myometrium

Addition		cGMP (pmol/mg protein)	cAMP (pmol/mg protein)	cAMP/cGMP
–		0.37 ± 0.05	5.0 ± 1.2	13
Theophylline (mM)	0.5	0.69 ± 0.09	5.4 ± 0.2	7.7
	10	1.90 ± 0.38	9.9 ± 0.7	5.2
MIX (μM)	55	1.25 ± 0.15	7.40 ± 0.3	5.9
	138	1.79 ± 0.36	9.01 ± 0.27	5.0
	276	2.72 ± 0.4		
	500	3.9 ± 0.66		
	1000	4.1 ± 0.55		

Incubation was carried out for 3 min in the absence or presence of theophylline or MIX at the indicated concentrations. Samples were extracted with TCA, cAMP and cGMP were separated by Dowex AG 1X8 formate column chromatography before each specific assay (see section 2). Values represent the means ± standard errors of 3–6 different experiments, each carried in duplicate

oxytocin, PGE_2, $PGF_{2\alpha}$, at a concentration range of 1–100 μM, as well as 100 mM K^+ did not detectably affect cGMP content of the rat myometrium incubated either in the absence or in the presence of 55 μM MIX. Under these conditions, with the exception of PGE_2 which markedly increased cAMP content, none of the contracting agents had any affect on basal levels of cAMP [4,5]. Epinephrine in a concentration range of 0.5–20 μM did not also exert any effect on myometrial cGMP content. Hence in either case where the muscle was promoted to contract by PGE_2 or to relax by epinephrine [5] there was an increase in the cAMP/cGMP ratio as compared to the control.

3.3. *Effect of guanylate cyclase activators*

Hydroxylamine is a potent activator of guanylate cyclase [18] and produces cGMP accumulation in slices of different tissues [8,9]. Results outlined in table 2 show that hydroxylamine and sodium nitroprusside exerted a significant increase in cGMP level of rat myometrium. Both effects were dose dependent and potentiated in the presence of MIX. Under such conditions, no effect on basal cAMP levels could be demonstrated.

Table 2
Effect of hydroxylamine and sodium nitroprusside on cGMP levels of rat myometrium

	cGMP pmol/mg protein	
Addition (mM)	- MIX	MIX (55 μM)
–	0.37 ± 0.05	1.44 ± 0.19
Hydroxylamine		
0.1	0.66 ± 0.1	(4.21– 3.46)
1	1.04 ± 0.1	8.8 ± 1.0
5	1.42 ± 0.24	(10.1 –12.0)
Sodium nitroprusside		
0.1	0.52 ± 0.05	
0.25	1.07 ± 0.29	9.72 ± 2.3
0.50	1.93 ± 0.28	
1.0	2.12 ± 0.21	16.5 ± 2.2

Myometrial strips were incubated in the absence or presence of 55 μM MIX for 3 min and incubation was further continued for 3 min in the presence of hydroxylamine or sodium nitroprusside at the indicated concentrations. Values represent the means ± standard errors of 8 different experiments except for the figures in parentheses which represent individual values of 2 separate experiments

3.4. *Role of Ca^{2+}*

The elevation of cGMP levels in smooth muscles in response to agents that stimulate contractions, in general has been described as a Ca^{2+}-dependent process [15,16,19]. The data depicted in fig.1 indicate that the basal turnover of cGMP in rat myometrium was not effectively changed by incubation in a Ca^{2+}-free medium containing 4 mM EGTA. Moreover the increase in cGMP due to 55 μM MIX as well as the stimulation induced by hydroxylamine could still be observed in these Ca^{2+}-deprived preparations. In fact, careful analysis of the results indicated that Ca^{2+} seems to exert a rather inhibitory effect. The same observation could be made for sodium nitroprusside (not shown).

Hence it is clear that in the rat myometrium, the Ca^{2+}-dependent mechanism, through which cGMP accumulation may occur, does not significantly operat under our experimental conditions. This may explain the lack of effect of carbamylcholine or high K^+ in increasing intracellular cGMP concentrations as compared to the stimulatory activities of these agents in a series of investigated tissues [19]. On the

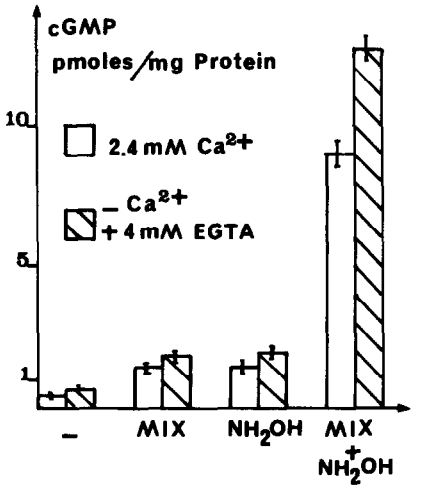

Fig.1. Role of Ca^{2+} on basal cGMP levels and on the stimulato effect of MIX and NH_2OH. Myometrial strips were incubated for 1 h in a normal (2.4 mM) Ca^{2+} medium or in a Ca^{2+}-free medium containing 4 mM EGTA. Tissues were transferred to a fresh corresponding medium with or without 55 μM MIX and in the absence or presence of 1 mM NH_2OH (see legend to table 2). Values represent means ± standard errors of 8 different experiments.

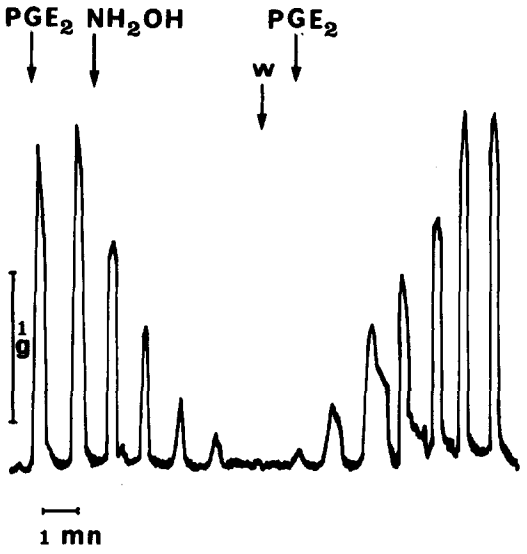

Fig.2. Tracing of isometric contractions of isolated rat uterus in the presence of PGE_2. Effect of hydroxylamine. The concentration of PGE_2 was 1.5 μM and NH_2OH 2 mM. w = washing with buffer solution.

other hand, significant guanylate cyclase activity appeared to be expressed in the myometrium through the alternate mechanism which does not require Ca^{2+} [8,9,19] but seems rather slightly inhibited by the divalent cation.

3.5. *Effect of hydroxylamine on uterine contractility*

Contractile activity of isolated uterine strips was measured as in [5]. When 2 mM NH_2OH was added to a uterine horn stimulated to contract by PGE_2, relaxation was observed (fig.2). This relaxing effect was reversible, with two successive washings restoring a normal contractile response to PGE_2. Hydroxylamine was also able to antagonize both oxytocin and $PGF_{2\alpha}$ evoked contractions. It is pertinent to point out that epinephrine induced relaxation of the myometrium is not accompanied by any change in tissue cGMP levels, but rather by an important elevation of intracellular cAMP.

In conclusion, the results obtained in the foregoing experiments, adding to those in [4–7], are not consistent with a general theory involving a direct causal relationship between changes in cAMP or cGMP and uterine relaxation or contraction. While neither cAMP nor cGMP could be considered as exclusive regulators, both cyclic nucleotides may still exert a modulatory activity at the level of alternative mechanisms (e.g., regulation of cellular concentrations and transport of Ca^{2+}) operating in the control of uterine contractility.

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