Variations in cGMP and cAMP levels in rat uterine smooth muscle induced by carbachol, $PGF_{2\alpha}$ and changes in ionic composition

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Schultz, Hardman, Schultz, Davis & Sutherland (1973) and Dunham, Haddox & Goldberg (1974) have shown recently that smooth muscle contraction induced by spasmogenic drugs is associated with an increase in cyclic guanosine 3'5'-monophosphate concentration (cGMP).

We have examined the influence of prostaglandin $F_{2\alpha}$ and carbachol (CCh) as well as of different experimental conditions on cAMP and cGMP levels in uterine smooth muscle.

$PGF_{2\alpha}$ 10^{-5} M, a concentration that produces a maximal contraction. There was no modification of cGMP or cAMP levels under $PGF_{2\alpha}$. CCh was applied to the muscle in concentrations varying from 10^{-6} to 10^{-3} M also for 45 s, 1, 2 and 5 min, without modifying cAMP or cGMP contents.

These results confirm and complete previous findings by Harbon & Clauser (1971). In order to explore further the role of these nucleotides in muscle cell function we have determined cAMP and cGMP levels after 10 min exposures to solutions of different ionic composition (Table 1). The reduction of extracellular Na^+ to less than 16 mM increases cAMP levels. This effect may be dependent on a reduction in the intracellular Na^+ concentration. The cGMP levels decrease in tissues treated with 129 mM K^+, either in the presence or in the absence of Ca^{2+}. This may be related to a stimulation of the Na^+-K^+-ATPase, since ouabain blocks the reduction of cGMP in high K^+

TABLE 1 Ionic content in extracellular solutions and cAMP and cGMP levels in rat myometrium

Test solution				*cAMP mM/mg protein*			*cGMP mM/mg protein*		
Na^+ mM	K^+ mM	Ca^{++} mM	*Ouabain mM*	*Control horn*	*Test horn*	*P <*	*Control horn*	*Test horn*	*P <*
112	4.66	0	0	2.6 ± 0.6 (1)	2.7 ± 0.50	NS	0.23 ± 0.04	0.24 ± 0.10	NS
112	4.66	1.5	1.0	2.9 ± 0.2	3.4 ± 0.30	NS	0.14 ± 0.02	0.14 ± 0.05	NS
16	129	1.5	0	2.9 ± 0.2	4.2 ± 0.30	0.001	0.14 ± 0.02	0.06 ± 0.01	0.001
16	129	1.5	1.0	3.4 ± 0.3*	5.1 ± 0.50	0.001	0.14* ± 0.05	0.12 ± 0.02	NS
16	129	0	0	2.6 ± 0.2	3.3 ± 0.02	0.050	0.22 ± 0.03	0.15 ± 0.01	0.050
87	29	1.5	0	3.3 ± 0.2	3.6 ± 0.20	NS	0.36 ± 0.08	0.26 ± 0.04	NS
87	29	0	0	2.3 ± 0.1	2.2 ± 0.10	NS	0.25 ± 0.03	0.16 ± 0.02	0.020
0**	4.66	1.5	0	3.0 ± 0.2	4.7 ± 0.60	0.020	0.37 ± 0.04	0.35 ± 0.03	NS
0**	4.66	0	0	2.4 ± 0.1	2.7 ± 0.10	NS	0.24 ± 0.03	0.19 ± 0.02	NS

(1) Mean ± standard error.
* 1 mM ouabain added to the control solution.
** Na replaced by Tris Cl.

Uterine horns were excised from virgin Wistar rats pretreated two days before the experiments with 0.5 mg/kg^{-1} day^{-1} of diethylstilboestrol dipropionate. Myometrial strips were prepared by eliminating mucosal and submucosal layers by dissection. The strips were equilibrated before use for at least 90 min in a normal Ringer solution. In each case, one uterine horn was used as a control and the contralateral horn for the experimental manipulation. cAMP and cGMP have been extracted and purified, and cyclic GMP levels determined, by the method of Murad, Manganiello & Vaughan (1971). The cAMP levels were measured by the Gilman technique (1970). The results are expressed in pM/mg of protein (Lowry, Rosebrough, Farr & Randall, 1951). The uterine strips were exposed during 45 s, 1, 2 and 5 min to solutions. The effects of low extracellular Ca^{2+} are paradoxical and have to be explored further.

In conclusion cAMP and cGMP do not seem to be involved in the mechanism of contraction of rat myometrium induced by drugs. They may participate in the regulation of some metabolic pathway.

We thank Dr P. Meyer for his helpful advice.

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The effects of cinanserin and phentolamine applied by microiontophoresis in the spinal cord

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Recent studies have shown that conditioning stimulation of the nucleus raphes medianus in the Fluothane anaesthetized rat increases the amplitude of the monosynaptic reflex evoked by dorsal root stimulation (Barasi & Roberts, 1974a). Intravenous L-tryptophan increased the effects of raphe stimulation, indicating that raphe stimulation may activate a pathway releasing 5-hydroxytryptamine (5-HT). More recent studies (Barasi & Roberts, 1974b) have shown that 5-HT applied by microiontophoresis into the ventral horn of the spinal cord increased the amplitude of the motoneurone field potential evoked by antidromic stimulation of the ventral roots. Conditioning stimulation of the nucleus raphes also increased the amplitude of the antidromic field potential. Intravenous (3-4 mg/kg) or iontophoretic (20-100 nA for 5-30 min) applications of cinanserin prevented the effects of raphe stimulation. The specificity of the blocking action of cinanserin remained uncertain, however, particularly as it was noted that a slight increase in dose caused a profound reduction in the amplitude of the unconditioned field potential. We have used noradrenaline as a control agonist to determine the specificity of the action of cinanserin.

Noradrenaline applied with iontophoretic currents between 50 and 100 nA increased the amplitude of the antidromically evoked field potential. Its effects were similar to those of 5-HT but the latency and response duration were longer. We recorded responses to alternate applications of 5-HT and noradrenaline and then superimposed an application of either cinanserin or phentolamine (20-75 nA for 5-20 min). Although both antagonists were capable of blocking responses to both agonists, this occurred with the higher currents of application and was usually but not always accompanied by reduction of the baseline field potential amplitude. The effects of cinanserin on 5-HT were more rapid and longer lasting than its effects on noradrenaline in 11 studies and were similar in two studies. Phentolamine had a greater effect on noradrenaline responses in seven studies and had a similar effect on noradrenaline and 5-HT responses in three studies. Occasionally, a very narrow dose range was identified when the antagonist reduced responses to the agonist without affecting the control agonist responses.

The facilitatory effects of conditioning stimulation of nucleus raphes were recorded during application of the antagonists. It was found that these responses could also be blocked by application of either antagonist but on every occasion the time course of the blockade closely followed the time course of the blockade of 5-HT.

We conclude from these studies that phentolamine and cinanserin can be used to differentiate between responses to noradrenaline and 5-HT in the spinal cord. The results lend support to the postulate that responses of motoneurones to 5-HT and conditioning stimulation of raphe are pharmacologically similar and differ from responses to noradrenaline.

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