Inhibition of Estrogen-Induced Elevation of Cyclic 3′, 5′-Adenosine Monophosphate in Rat Uterus

I. By *Beta*-Adrenergic Receptor-Blocking Drugs

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SUMMARY

The elevation of cyclic 3′,5′-AMP which occurs in the uterus of the ovariectomized rat within 30 sec following intravenous administration of estradiol-17β is subject to blockade by immediately prior injection by vein of the *beta*-adrenergic antagonists propranolol and, to a lesser extent, dichloroisoproterenol. Propranolol exhibited a slight agonistic effect, not hitherto recognized by other criteria; dichloroisoproterenol possessed this capacity to a striking degree, as had been anticipated. Bilateral adrenalectomy virtually abolished the inhibitory effect of dichloroisoproterenol on the response to estradiol-17β while interfering only slightly with that due to propranolol. The *alpha*-adrenergic blocking agents phentolamine and dibenzyline appeared inert by these criteria.

These data constitute the first observations correlating the effects of systemically administered adrenergic blocking agents with the cyclic 3′,5′-AMP response to estrogen in the intact uterus, and appear to support the suggestion that "*beta*-receptor" function may be closely associated with the adenyl cyclase system.

INTRODUCTION

It was demonstrated from these laboratories that adenosine 3′,5′-monophosphate, which arises from its precursor ATP through the action of the universally distributed enzyme adenyl cyclase, is responsive in its uterine locus to prevailing estrogen levels (1, 2). Thus, direct analysis of the intact uterus of the rat for endogenous cyclic AMP revealed that this nucleotide was depleted after ovariectomy. Its concentration was almost instantaneously restored, however, by intravenous administration of physiological quantities of estradiol-17β. This response was also elicited by diethylstilbestrol, but not by estradiol-17α, in accordance with established structural requirements for estrogenicity in the rat (2).

In an effort to analyze further the possible relationship between this phenomenon and the triggering of estrogenic activity, we have examined the capacity of various *beta*- and *alpha*-adrenergic receptor-blocking agents to interfere with elevation of uterine cyclic AMP due to estrogen. This approach was prompted by the known capacity of certain biogenic amines to stimulate the adenyl cyclase system (3–5), coupled with the demonstrated release of intrinsic histamine (6–8), serotonin (9), and epinephrine (10) during acute hormonal responses of various target organs, including the uterus (6, 8, 10). Moreover, precedent exists for the inhibition by certain adrenergic blocking drugs of both the cyclic

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AMP elevation as well as its metabolic correlates in the response of selected organs to their tropic hormones (cf. 11–13). Generally, these studies have been conducted on isolated structures or broken cell preparations *in vitro*.

In the course of the present investigations, which have been carried out *in vivo*, it has been determined that the acute elevation of uterine cyclic AMP due to estrogen is subject to varying degrees of inhibition by prior systemic treatment with certain *beta*-, but not *alpha*-, adrenergic receptor-blocking drugs.

MATERIALS AND METHODS

Most of the procedures utilized have been described earlier (2).

Female Sprague-Dawley rats, 6 weeks old and weighing about 150 g, were ovariectomized and maintained in a low-steroid environment under controlled temperature and lighting conditions for approximately 3 weeks. On the day of the experiment they were matched by body weight into control and experimental groups. In certain instances, adrenalectomy was carried out under light ether anesthesia 3 hr prior to administration of test compounds in order to obviate gross variations in circulating catecholamines and cortical steroids. In related studies, results of which are described elsewhere,[1] it has been demonstrated that exogenous cortisol and derivatives, but not deoxycorticosterone, are powerful inhibitors of uterine cyclic AMP elevation due to estrogen.

Estradiol-17β or its blank vehicle control solution was injected intravenously under light sodium pentobarbital (Nembutal) anesthesia as previously described (2). Adrenergic blocking agents were dissolved in 0.9% NaCl immediately prior to use, protected from light, and, unless otherwise indicated, given intravenously just before estrogen or control solution. These drugs included the *beta*-receptor antagonists dichlorisoproterenol and propranolol, and the *alpha*-adrenergic blocking agents phentolamine and phenoxybenzamine.[2] All intravenous injections were in the amount of 0.25 ml/100 g of body weight.

At precise intervals, usually 30 sec after hormone or control solution was injected, uteri were swiftly excised and plunged into liquid nitrogen. Preparation of the trichloracetic acid extracts of frozen, pulverized uteri pooled from three identically treated animals was carried out as described earlier (2).

Determination of cyclic AMP was conducted by a modification of the highly sensitive and precise method of Breckenridge (14), in which the tissue "blank" analysis was carried out in the presence of the specific phosphodiesterase[3] previously inactivated by boiling (2). Examples of the sensitivity and linearity (Fig. 1), reproducibility (Fig. 2), and additivity (Table 1) of this analytical method are given below.

RESULTS

Reliability of the method of assay. It is evident, from data adduced in Table 1 and in Figs. 1 and 2, and from results presented elsewhere (2), that the Breckenridge method as presently adapted lends itself favorably to the precise analysis of the moderately high levels of cyclic AMP encountered in the rat uterus. The reproducibility of these values in the ovariectomized rat from sample to sample (see also ref. 2), and on repeated analysis of the same sample, to-

[1] C. M. Szego and J. S. Davis, manuscript in preparation.

[2] Dichloroisoproterenol (DCI) was a gift from Eli Lilly and Company. Propranolol [Inderal; 1 - (isopropylamino) - 3 - (1-napthyloxy) - 2 - propanol hydrochloride] was generously provided by Ayerst Laboratories, Inc. Phentolamine [2-(*N*′-*p*-tolyl-*N*′-*m*-hydroxyphenylaminomethyl) - 2 - imidazoline] was kindly made available by the Ciba Pharmaceutical Corporation. Phenoxybenzamine (Dibenzyline; *N*-phenoxyisopropyl-*N*-benzyl-β-chloroethylamine hydrochloride) was contributed by Smith Kline & French Laboratories, Inc. Pronethalol [Nethalide; 2-isopropylamino-1-(2-naphthyl) ethanol hydrochloride] was generously donated by Imperial Chemical Industries, Ltd.

[3] The highly specific 3′,5′-cyclic mononucleotide phosphodiesterase was a generous gift of Dr. R. W. Butcher, whose cooperation is gratefully acknowledged.

TABLE 1

Simultaneous assay of mixtures of standard cyclic 3′,5′-AMP solutions and uterine extracts

All samples were analyzed simultaneously within a single experiment in duplicate, as indicated, along with appropriate blanks and additional standard cyclic AMP samples in trichloracetic acid. The two unknown samples were prepared as described in the text from uteri of ovariectomized animals 30 sec after intravenously administered vehicle control (C) or estradiol-17β, 11 μg/100 g of body weight (D), respectively. Cyclic AMP values given are for concentration in step *a* of the Breckenridge assay (2, 14). The volumes shown represent the amount of trichloracetic acid-free extract originating from standard or sample which was delivered to a total of 1 ml of a solution, also containing the hydrolytic enzymes and $MgCl_2$ in Tris-Cl buffer as specified by Breckenridge, prior to removal of 100-μl aliquots for incubation. Absolute values may be calculated as in the legend to Fig. 1.

	Content						3′,5′-AMP calculated			
	3′,5′-AMP-standard			Unknown						
Tube	Code	Concentration	Volume	Code	Volume	Net fluorescence	3′,5′-AMP found (from standard curve)	Standard (≅0.25 ml)	Unknown (≅0.50 ml)	Total	Additivity
		M × 10^{-7}	*ml*		*ml*		M × 10^{-7}		M × 10^{-7}		*% theoretical*
Separate analyses											
1	TC0	0	0.75			0	0	0			
2	TC0	0	0.75			0	0	0			
3	TC9	2.00	0.75			5.64	2.03	0.67			
4	TC9	2.00	0.75			5.54	1.98				
5				C30	0.75	1.49	0.53		0.35		
6				C30	0.75	1.46	0.53				
7				D56	0.75	6.55	2.35		1.57		
8				D56	0.75	6.58	2.36				
Mixtures											
9	TC0	0	0.25	C30	0.50	0.92	0.33	0.67	0.35	1.02	93.6
10	TC0	0	0.25	C30	0.50	0.95	0.34				
11	TC9	2.00	0.25	C30	0.50	2.59	0.95				
12	TC9	2.00	0.25	C30	0.50	2.69	0.96				
13	TC0	0	0.25	D56	0.50	4.27	1.54	0.67	1.57	2.24	96.4
14	TC0	0	0.25	D56	0.50	4.27	1.54				
15	TC9	2.00	0.25	D56	0.50	6.01	2.16				
16	TC9	2.00	0.25	D56	0.50	6.01	2.16				

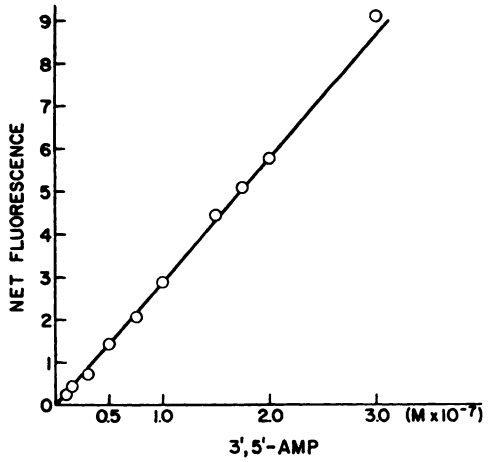

FIG. 1. *Composite curve of standards carried through the entire Breckenridge analytical procedure*

Analyses were conducted on known amounts of cyclic AMP, as described in the text, using an Aminco-Bowman spectrophotofluorometer for assessment of TPNH produced. Instrument parameters were as follows: xenon lamp 416-992; a potted 1P21 photomultiplier tube used at 1/5 scale sensitivity with slit arrangement No. 4; A = 340 mμ, F = 455 mμ (calibrated). Sensitivity was occasionally checked with quinine standards. Enzyme concentrations were such that the cycling rate was approximately 1800. Under the analytical conditions indicated, glucose-6-P at step *e* of the Breckenridge analytical procedure (2, 14), in a concentration of 3×10^{-6} M, would yield a net fluorescence value, in the arbitrary units herein utilized, of approximately 2.80. A TPNH fluorometric standard curve in the proportional range (14) was regularly run in parallel, together with its simultaneous molecular weight determination (ultraviolet), to calibrate the glucose-6-P yield in absolute units. Cyclic AMP (lots 50027 and 60046) was obtained from Calbiochem. Its freedom from trace adenosine phosphate contaminants, which were encountered in many other commercial preparations, was verified by paper chromatography (2). The concentrations of cyclic AMP shown are the levels present at step *a* (see also Table 1). These should be multiplied by 0.95 to correct for 1 mole of water, which was stably associated with the cyclic nucleotide under our conditions of storage. Apparent molecular weight was determined by the ultraviolet method, using a calibrated Gilford attachment to the Beckman DU spectrophotometer, and based on a molar extinction coefficient of 14,650 M^{-1} cm^{-1} at pH 7.0 (15). Each point represents the average of 2–18 determinations carried independently through the entire procedure in replicate experiments conducted during a 10-month period. The range is seen to be linear over a 30-fold variation in concentration. The deviations within these data were too low to be represented on the figure without undue scale expansion. For example, representative fluorometric values for 1×10^{-7} M cyclic AMP concentration at step *a* were 2.898 ± 0.022 (SEM) for 16 determinations. The results were unaffected by overnight freezing (−86°) of the diluted standard solutions following trichloracetic acid removal, as compared to immediate processing of the samples. Nor was there detectable deterioration of stock standard cyclic AMP in trichloracetic acid frozen in divided lots and analyzed at intervals over a 6-month period. The sensitivity of the assay, arbitrarily defined (14) as the concentration of 3',5'-AMP in the cycling step which gives a total fluorescence of twice the (uterine) tissue blank, was approximately 2×10^{-8} M. Control and experimental tissue blanks were indistinguishable. In the present procedure, subject to the correction factor indicated, an organ level of 1.17×10^{-6} M cyclic AMP corresponds to 1.0×10^{-7} M at step *a*; in terms of the amount actually being analyzed, there is present at this stage 1.0×10^{-11} mole of the nucleotide. Endogenous cyclic AMP values (± standard error) in mesometrial fat pad analyzed by the above method averaged 0.21 ± 0.02 (0.18, 0.21, 0.24) μmole/kg fresh weight and were thus entirely similar to those of control epididymal fat pads in the experiments of Butcher *et al.* (16–18) determined by totally independent procedures. The cyclic AMP content of mesometrial fat pad of the ovariectomized rat was unresponsive to estrogen treatment *in vivo*, as had been noted earlier (2) in the case of diaphragm. Thus, 30 sec after the intravenous administration of 1 μg of estradiol-17β per 100 g of body weight, concentration of the cyclic nucleotide in mesometrial fat pad averaged 0.22 ± 0.02 (0.20, 0.20, 0.26) μmole/kg fresh weight.

gether with prompt and organ-selective (see legend to Fig. 1) elevation in response to intravenously administered estrogen in physiological concentrations (Tables 1 and 2 and ref. 2), rendered it likely that inhibition of the hormonal effect on the cyclic nucleotide could readily be detected.

Lack of influence of adrenalectomy. For reasons indicated above, however, it was first necessary to determine whether potential antagonists to estrogenic action would function independently of adrenal activation. Hence, levels of uterine cyclic AMP were assayed in ovariectomized rats acutely deprived of their adrenals. Table 2 reveals

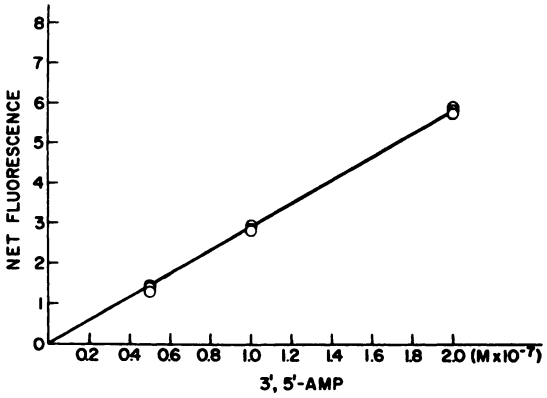

FIG. 2. *Reproducibility of the Breckenridge assay within a typical experiment*

Each point represents an individual determination of a known amount of cyclic AMP carried independently through the entire assay procedure as described in the text and Fig. 1 (see also Table 1).

that no significant alteration in endogenous cyclic AMP baselines or in responsiveness to administered estrogen was detectable in animals adrenalectomized 3 hr prior to the experiment. Moreover, in confirmation of earlier findings (2), response to a dose of estradiol-17β in the amount of 0.5 μg/100 g of body weight was maximal.

Suppressing effect of beta-adrenergic blockade. Table 3 shows that propranolol and, to a much lesser extent, DCI are capable of interfering with the uterine cyclic AMP accumulation response to estrogen. For example, propranolol in a dose of 0.1 mg/100 g of body weight (group 5) antagonized the activity of estradiol (compare the full-blown response to 0.5 μg/100 g of the estrogen in Table 2). This partial blockade was only slightly diminished in the absence of the adrenals (cf. group 7), in contrast to the lack of independence from adrenal mediation of the inhibitory capacity of DCI (see below). The suppressive influence of propranolol was statistically significant [$p < 0.01$, as calculated by the t-test for a series of paired observations (19)].

Both propranolol and DCI exhibited some agonistic effect of their own in the absence of estrogen (Table 2). Dobbs and this characteristic was somewhat more prominent in the case of DCI (groups 11, 12, and 13 vs. appropriate control values in Table 2) than it was in propranolol (groups 1–4). For propranolol, for example, the compound effect of antagonizing estrogen-induced cyclic AMP elevation while promoting that due to its own interaction with the receptor is reflected in the diminished effectiveness as an inhibitor of a doubled concentration of the drug in the presence of a fixed amount of estrogen (groups 6 and 8 vs. 5 and 7). The dose-dependent, direct stimulatory effect of propranolol is more readily revealed in the absence of estrogen

TABLE 2

Lack of influence of adrenalectomy on uterine cyclic AMP response to estradiol-17β

		3',5'-Cyclic AMP[a]			
		Fresh uterus		Dry uterus	
Group	Estradiol-17β	Control	Adrenalectomized[b]	Control	Adrenalectomized[b]
	μg/100 g body wt	*μmoles/kg*		*μmoles/kg*	
Vehicle control[c]		0.91, 1.06	0.99	3.70, 4.83	4.41
Estradiol-17β[d]	0.5	2.24, 2.55	2.13	9.42, 11.7	8.98
	1.0	1.88, 1.84	2.30	8.16, 8.38	9.67

[a] All values in this and subsequent tables are to be multiplied by a correction factor of 0.95 for conversion to absolute values, as indicated earlier (2). See also legend to Fig. 1.

[b] Adrenalectomy was carried out 3 hr prior to the experiment. All animals had been subjected to bilateral ovariectomy 3 weeks earlier and maintained thereafter as indicated in the text.

[c] Received blank vehicle at zero time. Uteri were removed to liquid nitrogen 30 sec later (see the text).

[d] Where indicated, estradiol-17β was administered intravenously at zero time (cf. 2).

TABLE 3

Influence of beta-adrenergic blocking agents on uterine cyclic AMP response to estradiol-17β

Group	Inhibitor dose	Estradiol-17β dose	Adrenalectomized[a]	3′,5′-Cyclic AMP[b]	
				Fresh uterus	Dry uterus
	mg/100 g	*μg/100 g*		*μmoles/kg*	
Propranolol					
1	0.1	—[c]	−	1.09	4.76
2	0.2	—	−	1.38	6.00
3	0.1	—	+	1.05	4.55
4	0.2	—	+	1.42	6.19
5	0.1	0.5	−	1.36	5.91
6	0.2	0.5	−	1.90	8.28
7	0.1	0.5	+	1.55, 1.51, 1.54	6.73, 7.32, 7.50
8	0.2	0.5	+	1.90	8.27
9	—	0.1	−	1.84	8.22
10	0.1	0.1	−	1.14	5.11
DCI					
11	1.0	—	−	1.16, 1.41	5.15, 6.03
12	1.5	—	−	1.12	4.87
13	1.0	—	+	1.53	6.59
14	1.0	0.5	−	1.52, 2.03	6.60, 8.72
15	1.5	0.5	−	1.73	7.50
16	0.5	0.5	+	2.16	9.11
17	1.0	0.5	+	2.07	8.91

[a] Adrenalectomy was carried out 3 hr prior to the experiment. All animals had been subjected to bilateral ovariectomy 3 weeks earlier and maintained thereafter as indicated in the text.

[b] See Tables 1 and 2. These values are to be compared with appropriate control groups in Table 2, wherein are presented data relevant to baselines in the presence and absence of the adrenals, with and without estrogen treatment (see also ref. 2).

[c] Where no hormone is indicated, blank vehicle, in an equivalent volume on a body weight basis, was administered intravenously at zero time. Tissue sampling was done 30 sec later.

(groups 1 and 3, 2 and 4, Table 3, vs. their respective controls in Table 2). When added to preparations *in vitro* or slowly infused *in vivo*, propranolol has exhibited no sympathomimetic activity detectable by other criteria (20, 21). The very short reaction time in the present experiments may have contributed to this positive finding.

Propranolol, at the dose of 0.1 mg/100 g of body weight, while effecting only partial blockade of the maximal dose of estradiol (0.5 μg/100 g of body weight), completely antagonized the effect of a submaximal concentration of hormone (group 10 vs. 9), reducing the uterine cyclic AMP concentration essentially to the level seen in the absence of estrogen (Table 2). Dobbs and Robison (22) had earlier observed blockade by propranolol of the cyclic AMP elevation produced in the isolated uterus on addition of isoproterenol to the organ bath.

The mild and inconsistent blocking effect of DCI on estrogen-activated cyclic AMP elevation in the uterus appeared to be diminished by prior adrenalectomy (groups 14 and 15 vs. 17, Table 3), possibly by virtue of removing endogenous cortical steroids whose secretion is likely to be elevated by the nonspecific stress due to administration of DCI at these dose levels. Sensitization to the agonistic effect of the blocking agent through reduction in circulating catecholamines, however, has not been ruled out as a contributory factor.

Pronethalol, structurally very similar to propranolol although possessing significantly less effectiveness as a *beta*-adrenergic blocking agent (20), was very poorly toler-

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TABLE 4

Influence of alpha-adrenergic blocking agents, alone and in combination with propranolol, on uterine cyclic AMP response to estradiol-17β

Group	Inhibitor dose		Propranolol dose (intravenous)	Estradiol-17β dose	Adrenalectomized[a]	3′,5′-Cyclic AMP[b]	
	Intraperitoneal	Intravenous				Fresh uterus	Dry uterus
	mg/100 g		*mg/100 g*	*μg/100 g*		*μmoles/kg*	
Phentolamine							
1	1.0			0.5	−	2.06	10.01
2	1.0			0.5	+	2.09	10.18
3	1.0		0.1		−	1.30	6.28
4	1.0		0.1	0.5	−	1.42, 1.45	6.91, 7.03
Phenoxybenzamine							
5		0.1			+	1.20	5.38
6		0.1		0.5	+	1.75	7.84
7		V.C.[c]		0.5	+	1.27	5.67

[a] See note in Tables 2 and 3.

[b] See note in Tables 1–3.

[c] Vehicle control. consisting of 0.2 ml of a stock solution (48.5 ml of absolute ethanol together with 0.53 ml of 37.5% HCl brought to a volume of 100 ml with propylene glycol) of the solvents used for phenoxybenzamine, diluted to 25 ml with 0.9% NaCl. The dose given, equivalent in volume to the phenoxybenzamine solution, was 0.25 ml/100 g of body weight.

ated when administered intravenously in doses of 1–2 mg/100 g of body weight, and investigation of its effectiveness in the present experiments was therefore not pursued. Acute toxicity following intravenous administration of pronethalol to mice has been attributed to a nonspecific action on the central nervous system rather than to *beta*-receptor blockade (cf. 20).

Lack of influence of alpha-adrenergic blockade. A limited number of experiments were conducted with *alpha*-adrenergic blocking agents. Table 4 reveals that phentolamine, administered intraperitoneally alone or in conjunction with a suboptimal ratio of propranolol given by vein, failed to block uterine cyclic AMP accumulation due to estrogen or to potentiate *beta*-adrenergic blockade. The apparent effectiveness of phenoxybenzamine was accounted for by the results with its vehicle control.

DISCUSSION

Inhibition by adrenergic blocking agents of metabolic responses to exogenous catecholamines is now a well-established phenomenon (21–28). Moreover, in some but not all instances in which alteration of steady-state cyclic AMP levels in intact cells appears to mediate these responses, it has been shown in isolated organ preparations that exposure of the receptors to selected antagonists (including substances other than adrenergic blocking agents) interferes not only with the metabolic correlates themselves, but also, and in the predicted direction, with the dynamics of metabolism of the cyclic nucleotide (cf. 11–13, 16, 22, 29).

The present experiments constitute the first demonstration that blockade of uterine *beta*-adrenergic receptors, hitherto characterized in this organ on the basis of their influence on motility alone (30), is associated with impairment of the cyclic AMP elevation due to estrogen. Thus, acute systemic treatment of ovariectomized rats with propranolol in moderately low doses abolished the effectiveness of submaximal concentrations of estradiol and significantly diminished the response to levels of the hormone, which, if unopposed, would have been maximal in eliciting cyclic AMP accumulation.[4] DCI exhibited manifestly less inhibitory influence than did propranolol, and its partial blocking action could be overcome, in contrast to that of propranolol, by prior adrenalectomy. However, in neither case did toxicity appear to play a role in the suppressive influence of the drug, for neither were there any signs of distress during or after the intravenous infusion, nor were the endogenous baselines of uterine cyclic AMP diminished in animals not receiving estrogen. Although propranolol has hitherto been described as a "pure" *beta*-antagonist with no agonistic influence per se (20), the present data reveal a slight dose-dependent effect of its own in promoting elevation of uterine cyclic AMP over the vehicle controls. This phenomenon was more pronounced for DCI, although at higher doses. The propensity of the latter drug to interact with *beta*-receptors as well as to antagonize the action of adrenergic compounds is well recognized (31–33). Thus, Powell and Slater have shown (31) that the capacity of the dichloro compound to antagonize relaxation of tone in isolated rat uterine strips previously exposed to epinephrine did not occur except with concentrations of the blocking agent which themselves promoted decreased motility.

In contrast to the relative effectiveness of *beta*-adrenergic blockade, the elevation of cyclic AMP due to estrogen was uninfluenced by prior systemic treatment with the *alpha*-adrenergic antagonists phentolamine and phenoxybenzamine. The blocking action of propranolol was, moreover, unmodified by combined treatment with phentolamine. These results were not unexpected, for

[4] After this manuscript was submitted, it was learned through the courtesy of Dr. G. Alan Robison of Vanderbilt University that he and Mr. James Dobbs have carried out similar experiments and confirmed, by an unrelated assay method, the elevation of uterine cyclic AMP in the ovariectomized rat following estrogen administration *in vivo* (2). Moreover, these workers independently achieved blockade of this response by prior systemic treatment with propranolol, as demonstrated in the present report (personal communication).

the positive correlation between cyclic AMP elevation and metabolic effects of catecholamines has been associated primarily with activation of *beta*-receptors (cf. 29). This generalization is borne out by indications (34) that *alpha*-adrenergic receptors in pancreatic islet tissue cells appear to inhibit the generation of cyclic AMP. Thus, it is evident that acute *beta*-adrenergic blockade, in contrast to *alpha* blockade, inhibits uterine cyclic AMP elevation due to estrogen. Whether this relationship is causal or fortuitous cannot be determined from the present data. These findings, however, appear to be in keeping with the general observations of Ahlquist (23, 30) on the predominance of *beta*-receptors, to the virtual exclusion of the *alpha*-type, in the myometrium of the nonpregnant rat (however, cf. 10, 35, 36).

The apparently discrepant findings of Levy and Tozzi (37), on the one hand, who showed that the inhibitory effect of catecholamines on rat myometrial contractility could be blocked by propranolol but not by phentolamine, and the observations of others (10, 35, 36), who have implicated *alpha*-adrenergic receptors in this organ, serve to illustrate the restrictive nature of the term "receptor," even within the motor framework of the original definition. Considerable light has been shed upon these conflicting data by the closely controlled studies of Tothill, which took into account the functional state of the organ in terms of its endocrine environmental history prior to removal to the assay bath (38; cf. also refs. 35, 39). Thus, adrenergic excitatory receptors, "inducible" by chronic exposure to large concentrations of estrogen, were postulated (38) in rat uteri 40–45 hr after massive doses of diethylstilbestrol, or in late pregnancy.

Further work is likely to reveal the degree to which the present conclusions are an oversimplification. Problems in interpretation involve lack of absolute specificity of interaction of drug and receptor in any case (39) and the existence of species and organ differences among receptors (13, 29, 35), to say nothing of the incomplete distinction, as yet, between metabolic and motor receptors (23, 40). Compounding these difficulties is the cellular inhomogeneity of the uterus. Beyond these problems there appears the need to identify specific metabolic receptors and determine to what degree they function in the uterine stimulation due to estrogen (see also ref. 36). Whether the present observations implicate an adrenergic component in estrogen action is open to further investigation. For example, the possibility has not been explored that epinephrine release from subcellular binding sites may accompany the demonstrated (6) site-specific discharge of histamine elicited by estradiol within seconds (8) of hormone administration. Nor has it yet been determined whether prior treatment with propranolol interferes with later evidence of estradiol stimulation of uterine metabolism as, indeed, it would be predicted to do if the cyclic AMP elevation is not merely a fortuitous accompaniment of generalized estrogen activation of target organ metabolic function, especially at membrane loci (8). In the case of endogenous adrenocortical hypersecretion (41) or exogenous cortisol administration (42) there is evidence of striking antagonism to estrogen action, while the initial elevation of cyclic AMP is essentially eliminated.[1] Deoxycorticosterone possessed neither capacity[1] (42). Since both propranolol (43, 44) and cortisol exhibit significant properties as membrane stabilizers, it is considered possible that both agents may function by a more generalized mechanism to overcome the effect of estrogen in promoting activation of membrane-localized adenyl cyclase in the uterus. The labilizing influence of estrogen on cytostructural barriers has been emphasized from these laboratories (2, 8). These considerations suggest that the inhibitory effects of propranolol upon estrogen-induced cyclic AMP elevation in the uterus may be exerted by a mechanism supplementary to pure *beta*-adrenergic receptor blockade (see also ref. 24).

Cyclic AMP exhibits significant estrogen-like action (8, 45), and, as suggested earlier (2, 8, 45; cf. also ref. 46), may serve to propagate certain metabolic responses of the

uterus to its tropic hormone. Whether the present observations can be related to the views of Robison *et al.* (13) regarding the virtual identity of both *alpha-* and *beta-*receptors with the adenyl cyclase system remains to be determined. Experiments in progress in these laboratories, using particulate preparations of rat uteri for the production of cyclic AMP *in vitro,* have revealed their reduced activity under circumstances of estrogen withdrawal.[5] These data, in keeping with earlier findings on estrogen-induced augmentation of uterine cyclic AMP levels *in vivo* (2), when taken in context with the present experiments, strongly suggest participation of the adenyl cyclase system, or of a membrane component close to it (22), in metabolic activation of the rat uterus due to estrogen.

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