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POSSIBLE REGULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN THE ADRENAL MEDULLA OF THE RAT BY A cAMP-DEPENDENT MECHANISM*

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Abstract— Ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, may be controlled by a cAMP-dependent mechanism. This hypothesis was investigated in the adrenal medulla of the rat. Exposure of rats to cold (4°, 2 hr) leads to increased cholinergic nerve transmission and to a 10- to 20-fold increase in cAMP levels in the medulla within 30 min. The cAMP level returned to normal within 2 hr of the initiation of cold exposure. Ornithine decarboxylase activity was elevated within 1 hr of cold exposure and by 4 hr was increased 10- to 20-fold. We also studied the effects of various drugs which were agonists and antagonists of the cAMP response to cold exposure in the medulla. Aminophylline (200 μmoles/kg), an inhibitor of phosphodiesterase activity, caused a large, rapid increase in the cAMP level followed by an increase in ornithine decarboxylase activity similar to that after cold exposure. Injection of a cholinomimetic drug, carbamylcholine (4.1 μmoles/kg), caused a 10- to 15-fold increase in cAMP within 20 min and a 10-fold elevation in ornithine decarboxylase activity within 2.5 hr. Pretreatment of the rat with the nicotinic receptor antagonist, mecamylamine (15 μmoles/kg), greatly reduced the carbamylcholine-induced rise in both cAMP levels and ornithine decarboxylase activity. Mecamylamine administered alone did not alter either cAMP levels or ornithine decarboxylase activity. Administration of reserpine (16 μmoles/kg) also resulted in an early rise in cAMP concentration in the adrenal medulla and a concomitant increase of ornithine decarboxylase activity. Cyclic AMP has been postulated to exert its effect on cellular metabolism via the activation of a cAMP-dependent protein kinase. Varying doses of reserpine from 1.6 to 16 μmoles/kg yielded a 1:1 relationship between the degree of activation of cAMP-dependent protein kinase(s) and the induction of ornithine decarboxylase. We feel that evidence from this and other laboratories supports the hypothesis that ornithine decarboxylase may be controlled by cAMP-dependent protein kinase(s).

Polyamines have been implicated in the regulation of growth processes in eukaryotes [1]. During periods of rapid growth, polyamine levels have been observed to increase dramatically, paralleling increases in RNA and protein synthesis [1–5]. Elevated polyamine levels are usually preceded by the induction of ornithine decarboxylase, the controlling enzyme in the polyamine biosynthetic pathway. The activity of ornithine decarboxylase can be increased rapidly in a variety of tissues in response to stimuli which result in an increase in RNA and protein synthesis. Little, however, is known about the mechanism by which ornithine decarboxylase is induced. The speed of this induction [2], as well as the many stimuli including hormones [6–9], methylxanthine derivatives [10] and dibutyryl cAMP [11–13] which are capable of inducing ornithine decarboxylase, suggests the possibility that ornithine decarboxylase is controlled by a cAMP-dependent mechanism. We chose to investigate this possibility in the adrenal medulla of the rat. The role of cAMP in the transynaptic induction of tyrosine hydroxylase in the adrenal medulla has been extensively studied [14–17] and forms the basis for the work presented here.

Cold exposure, aminophylline, carbamylcholine and reserpine all cause a rapid increase in medullary cAMP levels. We report that these stimuli also result in marked elevations in ornithine decarboxylase activity. Studies with actinomycin D and cycloheximide indicate that this increase in activity may involve *de novo* synthesis of the enzyme. The dose–response curve for reserpine indicated a 1:1 relationship between the increase in ornithine decarboxylase activity and the activation of cAMP-dependent protein kinase(s). Our results indicate that, in the adrenal medulla, the induction of ornithine decarboxylase may be controlled by cAMP-dependent protein kinase(s). We also discuss the possibility of a cAMP-mediated induction of ornithine decarboxylase in other tissues.

MATERIALS AND METHODS

The rats used in all experiments were male Sprague–Dawley (125–150 g). The activity of ornithine decarboxylase was determined in the 10,000 *g* supernatant of two to six freshly dissected adrenal medullae homogenized in 150–250 μl of assay media by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine

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(7.66 mCi/m-mole, New England Nuclear, Boston, Mass.). The assay was similar to that previously described [2, 7], except that the buffer used was 0.05 M sodium–potassium phosphate, pH 7.2, containing 1 mM dithiothreitol. The ornithine decarboxylase assays were performed at an L-ornithine concentration of 0.32 mM in a total volume of 100 μl. Saturated levels of substrate (+2 mM) yielded results similar to those reported here.

Cyclic AMP was determined in medullae of adrenal glands frozen within 10 sec of decapitation of the rat and separated from the adrenal cortex at 0–4° [14]. The medullae were homogenized in 0.4 N perchloric acid and cAMP separated from other acid-soluble nucleotides by aluminum oxide and Dowex chromatography [18]. The concentration of cAMP was determined by its ability to activate purified beef-heart cAMP-dependent protein kinase [19].

The activity of cAMP-dependent protein kinase in the adrenal medulla was determined in the 20,000 g supernatant according to the method of Guidotti *et al.* [20]. Kinase activity was assayed for 5 min at 30° in 170 mM sodium acetate, pH 6.0; 10 mM Mg acetate, pH 6.0; 25 mM NaF; 5 mM aminophylline; 50 μg of calf thymus histone (Schwarz/Mann); and 0.25 to 0.50 μCi of γ-^{32}P ATP (sp. act. 100 μCi/μmole). The degree of activation of cAMP-dependent protein kinase (i.e. the per cent of enzyme present as the free catalytic subunit not bound to the regulatory unit [21]) was expressed as the ratio of the activities measured in the absence and presence of saturating levels of cAMP (1 μM). Polyamine levels were determined from two to six medullae homogenized in 200 μl of 5% trichloroacetic acid, and an aliquot of the 10,000 g supernatant was analyzed on a Durrum amino acid analyzer [22].

RESULTS

The adrenal medulla consists of a uniform cell population of chromaffin cells whose cholinergic afferent nerves activate primarily nicotinic receptors [14]. The exposure of rats to cold (4°, 2 hr) leads to an increased rate of nerve transmission and elevated transmitter release from these afferent nerve terminals [23, 24]. Cold exposure has been shown previously to result in a rapid elevation in the level of cAMP in the medulla and in the induction of tyrosine hydroxylase by 12–24 hr [14, 24, 25]. Within 1 hr of the initiation of cold exposure, cAMP levels were elevated 10- to 15-fold above control values (Fig. 1). The concentration of cAMP then rapidly fell to its original level. The activity of ornithine decarboxylase was markedly elevated by 1 hr and was increased more than 10-fold by 4 hr of the initiation of cold exposure. We have shown [26] that severing of the splanchnic nerve (splanchnicotomy) prevents both this early increase in cAMP and the subsequent stimulation of ornithine decarboxylase.

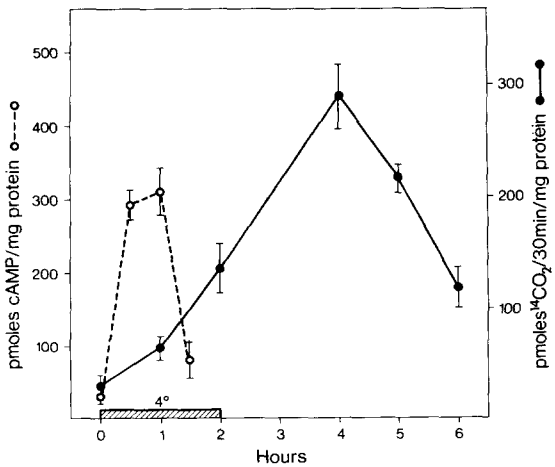

Fig. 1. Changes in cAMP concentration and ornithine decarboxylase activity in the adrenal medulla after cold exposure. Animals were immersed in water (25°) for 60 sec, placed in individual cages, and exposed to cold (4°) for 2 hr. After cold exposure, the animals were kept at room temperature. Cyclic AMP and ornithine decarboxylase were determined as described in Materials and Methods. Each point represents the mean ± S.E.M. of five to ten determinations.

Table 1. Changes in polyamine levels in the adrenal medulla after cold exposure*

Time (hr)	Putrescine	Spermidine	Spermine
	(pmoles/mg protein)		
0 (4°)	0.66	5.6	17.2
1 (4°)	0.89	5.2	16.5
2 (4°)	0.67	4.2	12.4
3	1.72	4.7	13.1
4	1.54	4.5	12.6
5	1.50	4.2	12.0
6	1.31	5.4	11.4
7	1.04	5.0	12.7

* Animals were immersed in water (25°, 60 sec), placed in individual cages at 4° for 2 hr, then removed and kept at room temperature. Polyamine levels were determined from the adrenal medulla of animals sacrificed at the times indicated after the initiation of cold exposure. The values shown are the mean of four determinations, not differing by more than 15 per cent.

The actual concentrations of polyamines in the adrenal medulla also changed after cold exposure (Table 1). The concentration of putrescine was elevated up to 2.5-fold between 3 and 6 hr after the initiation of cold exposure. While ornithine decarboxylase activity was falling by 5 hr (Fig. 1), the level of putrescine remained elevated until 6 hr after cold exposure. The concentrations of spermidine and spermine, however, actually decreased by 25 and 34% respectively. The concentration of spermine has also been observed to decrease by more than 50% in the regenerating rat liver [27]. The large amount of spermine relative to spermidine (3:1) is generally not found in mammalian tissues. Resting lymphocytes do, however, have spermine/spermidine ratios of 3:1 [28]. This high ratio may be characteristic of metabolically quiescent cells or of cells with a low probability of entering the cell cycle.

By using several drugs which are known to rapidly elevate cAMP levels in the medulla, we further investigated the relationship between cAMP and ornithine decarboxylase. Injection of the cholinomimetic drug, carbamylcholine (4.1 μmoles/kg) rapidly increased the concentration of cAMP in the adrenal medulla (Fig. 2). Cyclic AMP levels rose almost 20-fold within 45 min of injection. The ornithine decarboxylase acti-

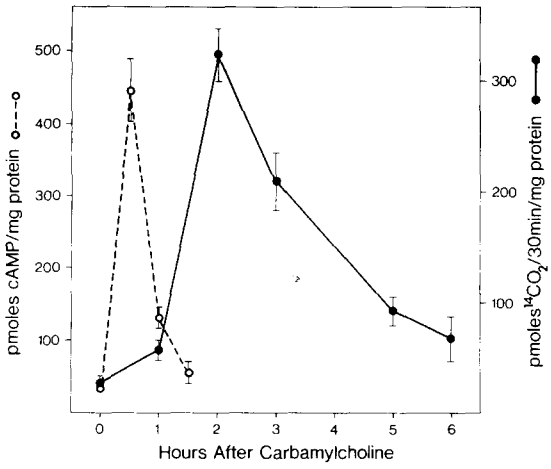

Fig. 2. Changes in cAMP concentration and ornithine decarboxylase activity in the adrenal medulla after injection of carbamylcholine. Carbamylcholine (4.1 μmoles/kg, i.p.) was injected in 0.9% NaCl, and cAMP and ornithine decarboxylase were determined at the times indicated. Each point represents the mean ± S.E.M. of five to ten determinations.

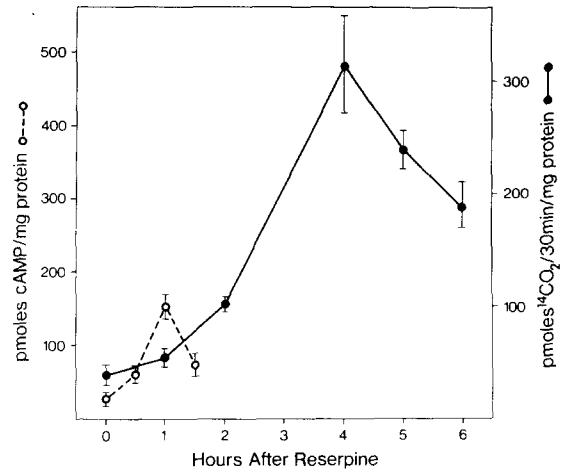

Fig. 4. Changes in cAMP concentration and ornithine decarboxylase activity after injection of reserpine. Reserpine (16 μmoles/kg, i.p.) was injected, and cAMP and ornithine decarboxylase were determined at the times indicate. Each point represents the mean ± S.E.M. of five to ten determinations.

vity was elevated by 1 hr and reached a maximum within 2 hr of injection (Fig. 2). The increase in ornithine decarboxylase activity which occurred between 1 and 2 hr after carbamylcholine administration was considerably more rapid than the elevation of ornithine decarboxylase activity after cold exposure.

Mecamylamine, a nicotinic receptor antagonist, is capable of blocking the effects of carbamylcholine on the medulla and can block the induction of tyrosine hydroxylase after cold exposure or carbamylcholine [14]. Mecamylamine (15 μmoles/kg) injected 15 min prior to carbamylcholine lowered the increased concentration of cAMP normally seen at 45 min to 100 pmoles cAMP/mg of protein and lowered the activity of ornithine decarboxylase at 2 hr to 220 pmoles of $^{14}CO_2$/30 min/mg of protein. Mecamylamine alone had no effect on cAMP or ornithine decarboxylase activity.

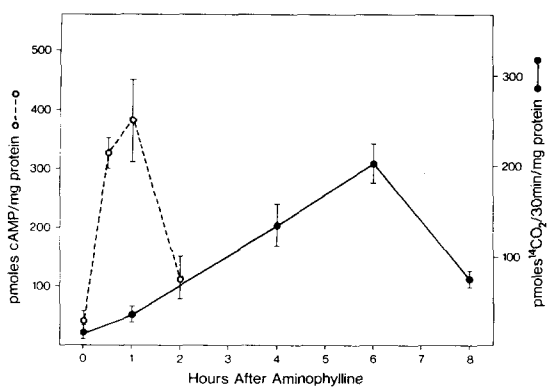

Fig. 3. Changes in cAMP concentration and ornithine decarboxylase activity after injection of aminophylline. Aminophylline (200 μmoles/kg, i.p.) was injected in 0.9% NaCl, and cAMP and ornithine decarboxylase were determined at the times indicated. Each point represents the mean ± S.E.M. of five to ten determinations.

Aminophylline, a methylxanthine derivative that is known to inhibit phosphodiesterases [29], also caused a large increase in cAMP 1 hr after injection (Fig. 3). Ornithine decarboxylase activity increased more slowly than after cold exposure and carbamylcholine, but reached a maximum 10-fold greater than control values within 6 hr (Fig. 3).

Reserpine has been shown to increase the level of cAMP in the adrenal medulla [17, 20]. The level of cAMP was elevated 5- to 6-fold within 1 hr of injection of reserpine (16 μmoles/kg) (Fig. 4). In a manner similar to that after cold exposure (Fig. 1), ornithine decarboxylase activity was increased at 1 hr and reached a maximum by 4 hr after administration of the drug.

Actinomycin D or cycloheximide at doses sufficient to block adrenal RNA or protein synthesis [30] was administered to animals at various times relative to the initiation of cold exposure or aminophylline injection (Table 2). Actinomycin D given 30 min prior to aminophylline or cold exposure blocked the large increase in ornithine decarboxylase activity normally seen 4.5 hr later (Figs. 1 and 3, Table 2). There was very little or no inhibition of ornithine decarboxylase when actinomycin D was injected at 1 or 2 hr. Cycloheximide, however, prevented the rise in ornithine decarboxylase activity seen at 4.5 hr at all times it was administered. These data are consistent with the rapid half-life (10–20 min) of ornithine decarboxylase observed in other tissues [1, 2]. Neither actinomycin D nor cycloheximide given 30 min before exposure to cold or aminophylline injection interfered with the rapid increase in cAMP concentration which normally occurred after these stimuli (Figs. 1 and 3).

Cyclic AMP has been postulated to exert its effects upon cellular metabolism through the activation of a cAMP-dependent protein kinase(s) [31]. If cAMP is involved in the induction of ornithine decarboxylase, then the degree of activation of the cAMP-dependent protein kinase(s) would be expected to reflect the extent of induction of ornithine decarboxy-

Table 2. Effect of inhibitors of RNA and protein synthesis on the increase in ODC activity in the adrenal medulla after cold exposure or aminophylline injection*

Time of injection (hr)			Relative ODC activity (% of control)	
Saline	Act D	Cycloheximide	Cold exposure	Aminophylline
0			1700	1300
	−0.5		200	180
	+1.0		1500	1050
	+2.0		1800	1400
		−0.5	80	95
		+1.0	59	85
		+3.5	98	89

* Actinomycin D (6 mg/kg, i.p.), cycloheximidine (50 mg/kg, i.p.) or saline alone was injected into rats at the indicated time relative to the initiation of cold exposure (4°, 2 hr) or to the injection of aminophylline (200 μmoles/kg, i.p.). ODC was assayed at 4.5 hr after the beginning of cold exposure or aminophylline injection. Ornithine decarboxylase activity is represented as per cent of the activity (i.e. no cold exposure or aminophylline) present in control animals receiving no stimulation. (Values shown are the average of six determinations not differing by more than 15 per cent).

lase. When the dose of reserpine was varied between 1.6 and 16 μmoles/kg, the activity of ornithine decarboxylase measured at 4 hr increased in a dose-dependent manner (Fig. 5). At concentrations of reserpine less than 1.6 μmoles/kg, there was no increase in ornithine decarboxylase activity above control values, and at concentrations greater than 16 μmoles/kg, no further elevation was observed. The activation of cAMP-dependent protein kinase(s) measured at its point of maximal activation (1 hr after reserpine injection [20]) occurred in a similar dose-dependent manner (Fig. 5). The time course for the activation of protein kinase and the stimulation of ornithine decarboxylase did not vary with the dose of reserpine.

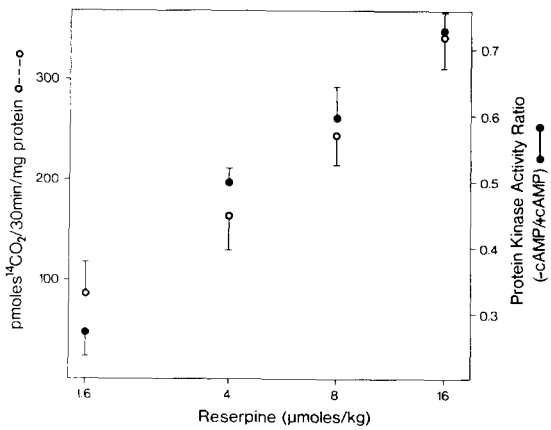

Fig. 5. Increase in ornithine decarboxylase activity and activation of cAMP-dependent protein kinase after varied doses of reserpine. Reserpine was injected at doses between 1.6 and 16 μmoles/kg, i.p. Ornithine decarboxylase activity was determined 4 hr after injection (Fig. 4). Cyclic AMP-dependent protein kinase was assayed as described in Materials and Methods at 1 hr after drug administration. Each point represents the mean ± S.E.M. of five to ten determinations.

DISCUSSION

There is a close temporal correlation between the elevation of cAMP and the increase in ornithine decarboxylase activity in the adrenal medulla of the rat. This elevation in ornithine decarboxylase activity after cold exposure or aminophylline administration, carbamylcholine administration, or reserpine administration was preceded in each instance by a 5-fold or greater increase in the concentration of cAMP (Figs. 1–4). The rise in cAMP was rapid, reaching a maximum in 30–60 min and returning to baseline by 1.5 to 2 hr. Ornithine decarboxylase activity was elevated in all cases within 1–2 hr and reached a maximum between 2 and 6 hr, depending upon the stimulus (Figs. 1–4). This relationship between cAMP and ornithine decarboxylase is strengthened by the observation that attenuation of the initial rise in cAMP by severing the splanchnic nerve before cold exposure [26] or by injecting mecamylamine prior to carbamylcholine significantly lowered the normally observed increase in ornithine decarboxylase activity. Ornithine decarboxylase and tyrosine hydroxylase are the only enzymes whose activities have been observed to increase after this rise in cAMP in the adrenal medulla [14–17].

Depending upon the stimulus used to induce ornithine decarboxylase in the adrenal medulla, there appears to be a difference in the time course of the increase in the activity. The maximal increase in ornithine decarboxylase activity occurs 2 hr after carbamylcholine (Fig. 2), 4 hr after cold exposure (Fig. 1) and reserpine (Fig. 4), and 6 hr after aminophylline administration (Fig. 3). There are several possible explanations of these differences. The increase in the concentration of cAMP is more rapid after carbamylcholine than after the other stimuli, perhaps accounting for the earlier increase in ornithine decarboxylase activity. It is possible that different stimuli might affect the turnover rate of ornithine decarboxylase, thus varying the rate of enzyme accumulation. The

time course of activation and deactivation of protein kinase in the adrenal medulla also might vary depending upon the particular stimulus. Recently, putrescine has been shown to inhibit the induction of ornithine decarboxylase [32]. It is possible that the different stimuli result in the accumulation of varying amounts of putrescine, which would modulate the induction of ornithine decarboxylase.

Although an increase in ornithine decarboxylase activity in the adrenal medulla was always preceded by elevated cAMP levels, there does not seem to be a direct correlation between the absolute increase in cAMP and the degree of increase in ornithine decarboxylase activity. Carbamylcholine resulted in a 15-fold elevation (Fig. 2) and reserpine in a 5-fold elevation in cAMP (Fig. 4), and both drugs caused the same increase in ornithine decarboxylase activity. If cAMP acts intracellularly through a protein kinase [31], the degree of activation of cAMP-dependent protein kinase(s) may be a better indicator of the physiological action of cAMP than is the actual concentration of the nucleotide. For example, follicle-stimulating hormone has been shown to exert its early biochemical effect upon the seminiferous tubules of the rat testis via a cAMP-dependent mechanism [33]. Significantly less hormone is required to maximally activate a cAMP-dependent protein kinase than is needed to result in maximal intracellular levels of cAMP [34]. Cyclic AMP-dependent protein kinase is activated at a concentration of follicle-stimulating hormone which produces a barely detectable increase in cAMP concentration [35]. Similar observations have been reported for the action of luteinizing hormone upon the testis [36] and of ACTH on the adrenal cortex [37]. It is for this reason that we investigated the dose-dependent increase in ornithine decarboxylase activity and of cAMP-dependent protein kinase activation. Figure 5 indicates that there is a close correlation between the degree of activation of cAMP-dependent protein kinase and the activity of ornithine decarboxylase. Guidotti *et al.* [20] originally showed a similar dose-dependent activation of cAMP-dependent protein kinase and induction of tyrosine hydroxylase in the medulla after reserpine. The level of induction of tyrosine transaminase in Reuber H35 cells also has been shown to depend upon the degree of activation of cAMP-dependent protein kinase(s) [38]. Such evidence strongly supports the possible cAMP-dependent control of enzyme activity.

The experiments with actinomycin D and cycloheximide seem to indicate that *de novo* synthesis of ornithine decarboxylase from newly transcribed mRNA is responsible for the increased activity of this enzyme seen in the medulla (Table 2). New RNA synthesis is required for at least 1 hr after tissue stimulation, after which induction of ornithine decarboxylase occurs in the absence of the synthesis of RNA. Inhibition of protein synthesis by cycloheximide blocks any increase in the activity of ornithine decarboxylase. Experiments with these inhibitors, implicating a transcriptional mechanism in the cAMP-dependent increase in ornithine decarboxylase activity, should be interpreted with caution since we are not measuring the actual rate of synthesis or amount of ornithine decarboxylase. Such studies await the preparation of specific antibodies to ornithine decarboxylase whose lability and poor antibody-titer thus far make this extremely difficult.

At present, the precise mechanism(s) whereby cAMP may regulate the synthesis of any specific protein has not been elucidated (for an extensive and well written review see Ref. 39). Regulation of gene activity by the phosphorylation of non-histone nuclear proteins by cAMP-dependent protein kinase(s) is one mechanism whereby cAMP might control enzyme synthesis at the transcriptional level [39].

Table 3. Correlation between cAMP levels, cAMP-dependent protein kinase activation, and ornithine decarboxylase activity*

Tissue	Stimulus	Cyclic AMP	Protein kinase	Ornithine decarboxylase	References
Liver, adrenals, BHK fibroblasts	Analogues of cAMP (dibutyrl cAMP)	↑		↑	11–13, 42
Liver	Regeneration	↑		↑	2, 43
Liver	Growth hormone		↑	↑	7, 8
Liver	Glucagon	↑	↑	↑	51–53
Uterus after castration	17β-Estradiol	↑		↑	44, 45
Salivary gland	Isoproterenol	↑		↑	49, 50
Liver	3-Methylcholanthrene, phenobarbital		↑	↑	40, 41
Adrenal medulla	Cold exposure	↑	↑	↑	20, 26
Adrenal medulla	Reserpine	↑	↑	↑	17, 20
Adrenal medulla	Carbamylcholine	↑		↑	14, 17
Adrenal cortex	ACTH	↑	↑	↑	13, 36
Liver, kidney, adrenal medulla, adrenal cortex, BHK fibroblasts	Methylxanthine derivatives	↑		↑	10, 11, 42
Chinese hamster V_{79} cells	Synchrony by mitotic selection	↑		↑	46
Insect pupae	Ecdysone	↑		↑	47, 48

* The systems listed illustrate instances in a variety of tissues when increased ornithine decarboxylase activity is preceded by or coincides with elevated cAMP concentration or activation of cAMP-dependent protein kinase(s). The table represents data compiled from this and other laboratories.

The evidence from our laboratory [10, 26] and others suggests that ornithine decarboxylase may be controlled by a cAMP-dependent mechanism in a variety of systems (Table 3). Growth hormone, which causes a large increase in ornithine decarboxylase activity in the rat liver [7, 8], to our knowledge, has never been shown to elevate the level of cAMP. We have found, however, that growth hormone does significantly activate cAMP-dependent protein kinase. We have also recently shown that phenobarbital and 3-methylcholanthrene, drugs which cause an increase in ornithine decarboxylase activity [40], also activate cAMP-dependent protein kinase [41]. It is possible that the activities of other enzymes besides ornithine decarboxylase are elevated after the stimuli are applied to the various tissues illustrated in Table 2. We know of no other enzyme, however, whose induction is preceded by an increase in cAMP concentration and/or activation of cAMP-dependent protein kinase in such a large variety of systems. This correlative evidence warrants further investigation into the generality of the cAMP dependency of the induction of ornithine decarboxylase and into the mechanism of this induction.

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