Gilford 2000 photometer); $\Delta\varepsilon$ between benzoylarginine and BAEE = 600 $M^{-1}$ $cm^{-1}$. Fluorescence was measured by Aminco-Bowman spectrofluorometer. The contribution from trypsin to the observed fluorescence was negligible under the experimental conditions used.

*Results and discussion.* Prococoonase obtained from *B. mori* was found to be homogeneous with a mol. wt of 27,000 on dodecylsulphate-polyacrylamide gel electrophoresis, and 32,000 on gel filtration. Upon activation by trypsin, 2 fragments are formed, which correspond to mol. wt of 21,000 and 10,000 on gel electrophoresis. The larger fragment is presumably cocoonase, for which a mol. wt of 20,000 has been previously reported[7]. With BAEE as substrate, the activated enzyme was found to have $k_{cat}$ of 22 $s^{-1}$ and $K_m$ of 1.0 mM at pH 7.8, 24 °C. These values may be compared with $k_{cat}$ of 36 $s^{-1}$ and $K_m$ of 0.057 mM reported for cocoonase from *Antheraea* pernyi[3], and $k_{cat}$ of 51 $s^{-1}$ and $K_m$ of 0.048 mM for cocoonase from *A. polyphemus*[5].

The intrinsic fluorescence spectrum of prococoonase, with excitation at 290 nm, is as shown in curve A of figure 1. The spectrum indicates main contribution from tryptophan residues in the protein. Upon activation by trypsin, the spectrum of the resulting mixture of cocoonase and the activation peptide is as shown in curve B of figure 1, with the same $\lambda_{max}$ of 342 nm, but a much reduced intensity. The quenching of fluorescence, to a total extent of 70% on complete activation, indicates that the tryptophan residues of prococoonase are in a different environment from those of the activated products. In contrast, inspite of extensive homologies between the cocoonases and trypsin[6], the fluorescence of trypsinogen is little changed on activation to trypsin[8]. Since there are only 2-3 tryptophan residues in prococoonase from various sources, none of which is lost from the enzyme on activation[6], the large quenching of fluorescence may reflect changes only in the microenvironment of these residues, and not necessarily protein conformational changes. Addition of 1 mM BAEE did not produce any change in the fluorescence spectrum of activated cocoonase.

The rate of activation of prococoonase by trypsin, as followed by activity assay with BAEE as substrate, is first order as shown in figure 2, in agreement with the result of Felsted et al.[5]. The rate of fluorescence quenching at 340 nm is also first order with respect to cocoonase, with the kinetic constant identical to that of enzyme activation for at least 2 half-lives (figures 2). It can be concluded that the change in environment of the fluophores occurs simultaneously with the formation of the active enzyme. The quenching of fluorescence can therefore be conveniently utilized to study the process of activation of this enzyme, especially when this is too rapid to follow accurately by activity assay.

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## Inhibition of prostaglandin-induced cyclic AMP accumulation in the rat anterior pituitary by alrestatin

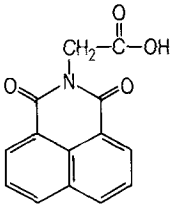
W. Lippmann[1]

*Biochemical Pharmacology Department, Ayerst Research Laboratories, P.O. Box 6115, Montreal (Quebec, Canada H3C 3JI), 15 August 1977*

*Summary.* Alrestatin at 25-1 × $10^{-4}$ M inhibited the accumulation of cyclic AMP induced by prostaglandin $E_2$, but not theophylline, in the rat anterior pituitary in vitro. Somatostatin, at lower concentrations, inhibited both; maximal inhibition of the prostaglandin effect was greater with alrestatin. As cyclic AMP is considered to be a mediator in induced-hormonal release, it appears from the present findings that alrestatin may be of potential use in altering hormonal release.

Cyclic AMP appears to be a mediator in prostaglandin-induced hormonal release. For growth hormone such a release has been observed in the rat anterior pituitary in vitro[2-5]. In vivo the plasma growth hormone levels are increased by administration of prostaglandin[6]. Prostaglandins and the cyclic nucleotide phosphodiesterase inhibitor theophylline increase both the accumulation of cyclic AMP and the release of growth hormone in the anterior pituitary in vitro[5,7,8]. The prostaglandin- and the theophylline-induced increases in cyclic AMP accumulation in the anterior pituitary in vitro are inhibited by somatostatin, the growth-hormone release-inhibiting hormone[7,8], as is the accompanying immunoreactive growth hormone and thyrotropin release[8]. Somatostatin also antagonizes the prostaglandin-, theophylline- and $N^6$-monobutyryl cyclic AMP-induced releases of immunoreactive growth hormone and thyrotropin from rat anterior pituitary cells in monolayer culture[9].

As with somatostatin, various of its analogs have been shown to be effective inhibitors of the prostaglandin-induced cyclic AMP accumulation in the anterior pituitary in vitro[10]. In the present studies alrestatin (figure), an aldose reductase inhibitor[11], has been found to antagonize the prostaglandin-induced increase in cyclic AMP in this system.



*Materials and methods.* The method employed in the determination of the accumulation of cyclic AMP in the anterior pituitary was based upon that reported previously[12,13] and was carried out as previously described[10,14]. The tissues were incubated, with shaking, for 60 min at 37 °C in an atmosphere of 5% $CO_2$ - 95% $O_2$ in 1.0 ml of Krebs Ringer bicarbonate buffer containing 11 mM D-glucose. The incu-

Table 1. Inhibition of prostaglandin-induced accumulation of cyclic AMP by alrestatin

| Addition | Final concentration ($M \times 10^{-4}$) | pmoles Cyclic AMP/anterior pituitary ± SE | −Control | Percent inhibition |
|---|---|---|---|---|
| None | | 8.3 ± 0.6 | | |
| Somatostatin | 0.001 | 9.4 ± 0.5 | | |
| Alrestatin | 5.0 | 8.0 ± 0.4 | | |
| $PGE_2$ | 0.01 | 50.4 ± 5.2 | 42.1 ± 5.2 | |
| Somatostatin + $PGE_2$ | 0.005 | 33.8 ± 1.1 | 24.4 ± 1.1* | 42 |
| | 0.001 | 25.9 ± 2.8 | 16.5 ± 2.8** | 61 |
| | 0.0002 | 46.4 ± 7.8 | 37.0 ± 7.5 | 12 |
| Alrestatin + $PGE_2$ | 25.0 | 8.8 ± 0.8 | 0.8 ± 0.3*** | 98 |
| | 5.0 | 19.3 ± 2.2 | 11.3 ± 2.2** | 73 |
| | 1.0 | 31.9 ± 4.8 | 23.9 ± 4.8* | 43 |
| | 0.05 | 46.2 ± 5.9 | 38.2 ± 5.9 | 9 |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

bation medium was then replaced by fresh buffer and glucose and the test compound; after a further 2-min incubation, 20 µl vehicle, prostaglandin $E_2$ ($PGE_2$) or theophylline was added for the incubation period of 4 min. The alrestatin (employed as its sodium salt) was prepared by Dr K. Sestanj, Chemistry Department, Ayerst Research Laboratories, and somatostatin by Immer et al.[15]. $PGE_2$ was purchased from ONO Pharmaceutical Co. and theophylline (monoethanolamine) from K & K Laboratories.

*Results.* Somatostatin exhibited the ability to inhibit, in a concentration-dependent manner, the increase in cyclic AMP produced by $PGE_2$ ($1 \times 10^{-6}$ M) in the rat anterior pituitary (table 1). Alrestatin also antagonized the increase being effective in a concentration range of $25\text{-}1 \times 10^{-4}$ M. Alrestatin exhibited a higher inhibition, i.e. a 98%-inhibition at a level 5 times that giving a 73%-inhibition while the inhibition after somatostatin under comparable conditions, i.e. 61%, was not increased. Neither alrestatin nor somatostatin antagonized the basal cyclic AMP accumulation.

As with $PGE_2$ an increase in cyclic AMP accumulation was produced by theophylline (table 2). The rise in cyclic AMP was concentration-dependent at $1\text{-}5 \times 10^{-2}$ M with the increase being about 2fold at $1 \times 10^{-2}$ M (table 2); this latter level of theophylline was employed in the subsequent studies.

Alrestatin was ineffective in preventing the theophylline-induced ($1 \times 10^{-2}$ M) increase in cyclic AMP at concentrations of $1\text{-}5 \times 10^{-4}$ M, a range in which it antagonized the $PGE_2$-induced ($1 \times 10^{-6}$ M) increase in cyclic AMP (table 3). In contrast, somatostatin inhibited the theophylline-induced cyclic AMP accumulation at a concentration similar to that at which it antagonized the $PGE_2$-induced increase.

Table 2. Stimulation of cyclic AMP accumulation by theophylline

| Addition | Final concentration ($M \times 10^{-2}$) | pmoles Cyclic AMP/anterior pituitary ± SE |
|---|---|---|
| None | | 6.8 ± 0.7 |
| Theophylline | 5.0 | 27.4 ± 1.0* |
| | 1.0 | 14.3 ± 1.1** |
| | 0.2 | 7.9 ± 0.8 |

*$p < 0.01$; **$p < 0.001$.

*Discussion.* The present findings demonstrate that alrestatin is an effective antagonist of cyclic AMP accumulation. Alrestatin differs in action from somatostatin in antagonizing induced-increase in cyclic AMP accumulation in the rat anterior pituitary in vitro since it inhibits only the prostaglandin-induced increase, and not that of theophylline, while somatostatin[7,8] inhibits both, under their respective comparable conditions.

The maximum inhibition obtained with somatostatin was about 50% and this is in accord with the findings of others[8]. As the growth hormone (and thyrotropin)-secreting cells comprise about this amount of the adenohypophyseal cells, complete inhibition of the cyclic AMP accumulation in these cells appears to have occurred[8]. In contrast to somatostatin, alrestatin caused inhibition greater than 50%. These findings would be consistent with the suggested different mechanism of action for alrestatin.

Alrestatin, through an action at the prostaglandin receptor on the pituitary somatotrope linked to an adenyl cyclase-cyclic AMP system, could cause the reduction of the activity. Such an action can be achieved with an agent more structurally-related to prostaglandin, i.e., the prostaglandin analog 7-oxa-13-prostynoic acid[16] or 13-hydroxy-9-oxo-prost-14-ynoic acid[17]. In addition to a possible inhibitory action on the rate of cyclic AMP synthesis, alrestatin could also act to increase the rate of destruction of the cyclic AMP through stimulation of the cyclic nucleotide phosphodiesterase activity; various agents are known to increase phosphodiesterase activity[18].

Alrestatin exhibited appreciable activity in the present

Table 3. Effect of alrestatin on theophylline-induced accumulation of cyclic AMP

| Addition | Final concentration ($M \times 10^{-4}$) | pmoles Cyclic AMP/anterior pituitary ± SE | − Control | Percent inhibition |
|---|---|---|---|---|
| None | | 7.3 ± 0.4 | | |
| Theophylline (T) | 100.0 | 17.3 ± 0.6 | 9.9 ± 0.6 | |
| Alrestatin + T | 5.0 | 18.3 ± 0.6 | 11.0 ± 0.6 | - |
| | 1.0 | 17.9 ± 0.9 | 10.6 ± 0.9 | - |
| None | | 7.7 ± 0.7 | | |
| T | 100.0 | 20.7 ± 1.1 | 13.0 ± 1.1 | |
| Somatostatin + T | 0.001 | 13.7 ± 0.7 | 6.0 ± 0.7* | 54 |
| | 0.0002 | 19.5 ± 2.1 | 11.8 ± 2.1 | 19 |

*$p < 0.01$.

studies at the $10^{-4}$ M concentration. For inhibition of lens aldose reductase (bovine) in vitro, levels of $10^{-5}$ M and $10^{-6}$ M were effective, and $10^{-4}$ M was inhibitory in a lens tissue culture system (rabbit)[11]. In the system employed in the present studies, the prostaglandin analog 13-hydroxy-9-oxoprost-14-ynoic acid was an effective antagonist at $5 \times 10^{-4}$ M, but not at $5 \times 10^{-5}$ M[17].

Due to its ability to antagonize the action of prostaglandin to cause cyclic AMP accumulation, alrestatin is of potential relevance in various areas depending upon its specificity of action. In this regard the 7-oxa-13-prostynoic acid inhibits the release of various hormones, e.g., adrenocorticotropic and thyroid stimulating hormones by their respective releasing factors[19] and growth hormone by prostaglandin[16] in hemipituitaries in vitro. Alrestatin is of interest with respect to its ability to inhibit the aldose reductase which results in decreased levels of polyols in galactosemic and diabetic rats[11]. Among the possible effects of alrestatin on hormone release is that on glucagon release. Such an action would be independent of aldose reductase inhibition but could be complementary.

The specificity of the antagonism by alrestatin is of interest in relation to the different actions of prostaglandins, species and tissues, and other agonists as well as are the other possible physiological and pharmacological activities of alrestatin.

It is of importance that somatostatin has been demonstrated to antagonize various hormonal secretions, e.g. in humans, among these being glucagon, insulin, growth hormone, prolactin and gastrin[20]. Further, gastric acid secretion has been observed to be antagonized by somatostatin in humans[20]. Recently, somatostatin has been found to inhibit gastric acid secretion in the rat[21]. In this regard, it is of interest that alrestatin has also been found to inhibit gastric acid secretion in the rat[22].

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## Possible relationship between packed sperm volume and egg mass in domestic fowl

H.L. Marks

*Agricultural Research Service, U.S. Department of Agriculture, Southern Regional Poultry Breeding Project, UGA, 107 Livestock-Poultry, Bldg., Athens (Georgia 30602, USA), 25 August 1977*

*Summary.* Packed sperm volume (PSV) values of male chickens have been observed to parallel the daily egg mass values of laying hens. Selection progress was greater when selection was for PSV and egg mass than for egg mass alone.

Some important economic traits in livestock production such as milk yield in dairy cattle and egg production in the domestic fowl are sex limited. This limitation precludes reliable means other than progeny and sib tests of estimating the corresponding genotype in the opposite sex. Consequently, the genetic progress for the improvement of these traits is less than that of traits measurable in both sexes. This situation often applies to reproductive traits and results in a lower selection intensity for the sex-limited trait. Egg production in the domestic fowl is an example of a trait that fails to respond to mass selection after initial gains, and further improvement requires either progeny testing or sib selection[1]. Because these selection systems are more complex and time consuming than mass selection, the development of a procedure involving individual phenotypic selection in both the male and female would be desirable.

We have initiated studies to investigate possible methods of improving reproductive efficiency in laying hens by applying selection pressure to both males and females. The advantage of using egg mass (egg number × egg weight) as a biological selection index has received little investigation[2]. The establishment of a parameter such as egg mass as the criterion for selection to improve egg production efficiency may allow the identification of a possible corollary in the male. If egg mass is defined as the total reproductive mass produced by the female (composite of both egg size and number), then the packed cell volume of semen could represent a corresponding unit in the male, assuming that gonads of both males and females are subject to control by common neuroendocrine systems. Although little attention has been devoted to the male's role in improving egg production of the domestic fowl, semen production and egg production are related in White Leghorns as evidenced by selection for high and low