

CHANGES IN THE ACTIVITY OF AN ALKALINE PHOSPHATASE IN PREPUBERAL MOUSE OVARIES

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SUMMARY

An alkaline phosphatase which occurs in immature mouse ovaries has been studied. Its activity was enhanced by gonadotrophins with luteinizing hormone (LH) activity. In control mice its level fluctuated markedly with age, following the pattern of serum LH concentration. The capacity of the ovary to produce more of this enzyme in response to injected hormone also varied with age. During a refractory period injected gonadotrophin induced the synthesis of much oestrogen, whereas during a responsive period much less oestrogen was produced. The possible role of this enzyme is discussed.

INTRODUCTION

An alkaline phosphatase may be demonstrated in immature mouse ovaries by standard histochemical methods (BEN-OR, 1963 ; BRANDAU, 1970 ; KENT and RYLE, 1975). Another alkaline phosphatase, which we have termed alkaline phosphatase I (Alk.Ph.I) is also demonstrable in such ovaries (BRAMLEY, 1974), but its substrate requirements, pH optimum and susceptibility to various inhibitors are quite distinct (table 1). Both enzymes occur in the follicular theca, stroma and interstitial tissue of the ovary (KENT and RYLE, 1975 ; BRAMLEY and KENT, in preparation) and, at certain stages of development, the activity of each is augmented by injected gonadotrophins (BRAMLEY, in press ; BRAMLEY and KENT, in preparation).

In homogenates of ovaries from 21 day old mice, of the CFW strain, the specific activity of Alk.Ph.I is low. However, treatment of the mice with human chorionic gonadotrophin (hCG) or human luteinizing hormone (hLH) results in a sharp increase in specific activity and also a marked rise in the affinity with which it binds to the

substrate (K_m). These changes do not become apparent until 6-8 h after injection and only reach maximal levels after 24 h (BRAMLEY, 1974). This distinct activity has been designated alkaline phosphatase Ib (Alk.Ph.Ib). Its substrate requirements, pH optimum, inhibitor sensitivity, etc. are similar to those for Alk.Ph.I. It is induced specifically by gonadotrophins with LH activity. Nevertheless, although human follicle stimulating hormone alone is ineffective, it augments the effects of hLH and hCG when administered simultaneously (BRAMLEY, 1974).

TABLE I

Alkaline phosphatase activities in mouse ovaries which respond to gonadotrophins

	Demonstrated by Gomori's calcium cobalt method	Alkaline phosphatase I
Optimal pH	9.4	10.4
Substrates	Wide range of organic substrates hydrolyzed	<i>p</i> -nitrophenyl phosphate and naphthylphosphate hydrolyzed most rapidly
Ca ²⁺	87mM CaCl ₂ in medium	Ca ²⁺ inhibits
Iodoacetate	Inactivates	No effect
N-ethylmaleimide	Inactivates	No effect
EDTA + Glycine	No effect	Inactivates

Although Alk.Ph.I and Alk.Ph.Ib are remarkably similar enzymically, their chemical and physical properties are quite distinct. Studies of these properties suggest that the weakly active Alk.Ph.I is composed of the highly active Alk.Ph.Ib associated with an inhibitory protein, which is rich in sialic acid. Gonadotrophin treatment *in vivo* appears to dissociate this complex and to allow full expression of the Alk.Ph.Ib activity (BRAMLEY, unpublished data).

AGE-RELATED VARIATIONS IN ALKALINE PHOSPHATASE ACTIVITY

During normal development the ovarian content of Alk.Ph.Ib fluctuates markedly (BRAMLEY, in press). The changes in substrate binding affinity (K_m) follow the pattern of changes in the serum content of LH (DULLAART, KENT and RYLE, 1975). There is an initial increase until about 6 days after birth. A high level is maintained until 12 days, then the substrate binding affinity declines rapidly. Thereafter it remains low until shortly before puberty. There is, in addition, on Day 20 after birth, a rise in specific activity with no associated marked increase in binding affinity,

suggesting an enhanced level of Alk.Ph.I but not of Alk.Ph.Ib. This level of specific activity is maintained until Day 35 after which Alk.Ph.Ib increases progressively until ovulation occurs at Day 40 to 45.

The capacity to respond to exogenous gonadotrophin also varies dramatically with age (BRAMLEY, in press). There is no response to injections of hCG (5 iu/10 g body weight) until 18 days after birth. Before Day 9 high endogenous LH levels may already be inducing a maximal response. From Day 10 to 16, however, serum LH levels are declining or low and some other factor must be limiting the capacity to respond to exogenous hormone. From Day 18 to 29 both specific activity and substrate binding affinity are progressively and markedly enhanced by hCG, indicating increasing activation of Alk.Ph.Ib. A further somewhat refractory period follows from Day 30 to 35 but subsequently the capacity to respond again increases progressively until the time of the first ovulation.

DISCUSSION

Assuming that Alk.Ph.Ib is derived from Alk.Ph.I, the fluctuations in the capacity to respond to injected hCG may reflect variations in the ovarian content of the latter. What controls the supply of Alk.Ph.I? DÖHLER and WUTTKE (1974), using rats, found age-related changes in serum prolactin levels which closely parallel the mouse ovarian alkaline phosphatase response to hCG. This suggests that prolactin may control the ovarian supply of Alk. Ph.I available for the production of Alk.Ph.Ib.

Finally what is the function of Alk.Ph.Ib ? Between Days 30 and 33, the second refractory period, hCG injections induce rapid oestrogen secretion and a massive increase in uterine weight. In contrast between Days 22 and 29, when hCG markedly enhances the activity of Alk.Ph.Ib, its effects on oestrogen secretion and uterine weight are much less (BRAMLEY, in press). Thus much oestrogen is produced in response to hCG when the enhancement of Alk.Ph.Ib activity is weak and vice versa. It is possible that the function of Alk.Ph.Ib, induced by gonadotrophins, is to switch off the oestrogen synthesizing mechanism.

LH and hCG rapidly activate mouse ovarian adenylyl cyclase to produce cyclic AMP (KUEHL *et al.*, 1970). This in turn may stimulate protein kinases (KUO *et al.*, 1970) to phosphorylate certain proteins essential for steroidogenesis. The same hormones activate, *but more slowly*, Alk.Ph.I. to Alk.Ph.Ib which may dephosphorylate the same proteins, so serving as a regulator to prevent excessive oestrogenic stimulation at certain stages of development. The overall effects of LH during normal prepubertal growth may thus fluctuate, depending not only on the serum concentration of this hormone but also on the varying supply of Alk.Ph.I and on its control.

RÉSUMÉ

MODIFICATIONS DE L'ACTIVITÉ DE LA PHOSPHATASE ALCALINE
DANS LES OVAIRES DE SOURIS PRÉPUBÈRE

Une phosphatase alcaline apparaît dans l'ovaire de Souris immature. Son activité est augmentée par les gonadotropines à activité LH. Son niveau varie fortement avec l'âge en fonction de l'évolution de la LH plasmatique. La capacité de l'ovaire à produire davantage de cette enzyme en réponse à une gonadotropine exogène varie également avec l'âge : on n'observe aucune réponse jusqu'à 18 jours après la naissance et entre 30 et 35 jours la réponse est plus faible qu'à 28-29 jours ou après 35 jours.

L'injection de gonadotropine pendant une période réfractaire augmente la synthèse d'estrogène, si l'injection est faite pendant une période sensible, la synthèse d'estrogène est moins importante. Le rôle de cette phosphatase induite par les gonadotropines pourrait être de changer le mécanisme de synthèse des estrogènes.

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