CHOLINESTERASE ACTIVITY IN THE CORPUS LUTEUM OF THE SHEEP AND PIG

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Summary: The acetylcholinesterase (AChE) activity in the corpora lutea of ovaries from sheep has been examined at different stages of pregnancy and the oestrous cycle, by both quantitative and histochemical techniques. The enzyme activity rose during the early part of pregnancy and then levelled out; it appeared to fall sharply at term. During the oestrous cycle the AChE activity was much lower but showed time-related changes. A few results from pigs and cows are included. The cow corpora lutea, unlike those of the sheep and pig contained butyrylcholinesterase (BuChE) as well as AChE. The results are discussed in terms of the possible function of non-neuronal cholinesterases.

Key words: acetylcholinesterase, corpus luteum, sheep, pig, pregnancy, cow, oestrous cycle

INTRODUCTION

The occurrence of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in non-nervous tissue is well documented but their role at such sites is unknown (7). Because, in many species, the enzymes are present in various glandular structures including salivary, intestinal and sweat glands, and the pancreas, thyroid and parathyroid glands, it has been suggested that AChE and BuChE could play some part in secretory activity but direct evidence to support this idea is lacking. Similarly, attempts to show that the cholinesterase found in the erythrocyte or platelet membrane and in the brain capillaries of some species is involved in the control of membrane permeability have not yielded convincing proof.

This paper describes the presence of acetylcholinesterase in another secretory structure, the corpus luteum (CL) of the sheep ovary. A few data obtained from pig and cow ovaries are included.

Results of the study suggest that the corpus luteum may be a useful preparation in which to investigate more fully whether or not AChE is involved in the synthesis or release of glandular secretions.

MATERIALS AND METHODS

Sheep: Ovaries were obtained, at different known stages of the oestrous cycle and pregnancy, from Clun Forest, Soay and Welsh Mountain ewes (see Figs. 1, 2). The cycle in the sheep takes 16 days and pregnancy lasts 142-148 days. Most ovaries came from animals which had been anaesthetized with sodium pentobarbitone and exsanguinated but some were removed under halothane anaesthesia. All CL present were analysed.

Pigs: A limited number of ovaries (see Fig. 3) from Large White sows were examined. With the exception of those from 2 animals, obtained at known times in the oestrous cycle (this takes 21 days), all ovaries were taken from pregnant pigs, the majority being obtained between 12 and 18 days of pregnancy, which in this herd lasts for about 114 days. The pigs were killed with a captive bolt pistol and exsanguinated. If both ovaries were available, one CL was taken from each, otherwise 2 CL were taken from the same ovary.

Cows: One corpus luteum was obtained from each of 3 cows when respectively 29, 33 and 66 days pregnant. The cows had been shot with a captive bolt pistol and exsanguinated.

Acetylcholinesterase analysis and histochemistry: Ovaries were put on ice on removal and kept cold while the corpora lutea were dissected out. After each CL had been weighed a sample (20-50% of the whole) was sliced off for histochemical examination. The remainder was reweighed, frozen in liquid nitrogen, pulverized in a percussion homogenizer and suspended in 0.32*M* sucrose at a concentration of 100 *mg/ml*; the suspension was further homogenized in a teflon-in-glass homogenizer. All homogenates were deep frozen (-20°*C*) for at least 24 hr before being assayed for cholinesterase activity by the Ellman technique (2). Preliminary experiments with different substrate-inhibitor combinations showed that in the sheep and pig almost all the cholinesterase activity of the CL was attributable to AChE but in the cow BuChE activity was appreciable. Routinely, therefore, sheep and pig corpora were assayed for AChE only but the cow corpora were assayed for BuChE as well. For the determination of AChE activity, acetylthiocholine iodide (AcThCh; Sigma) was used as substrate with 0.1 *mM* ethopropazine hydrochloride (May and Baker Ltd) to inhibit BuChE activity. Butyrylthiocholine iodide (BuThCh; Sigma), combined with 0.5 *mM* BW 284C51 (Wellcome Reagents Ltd) to inhibit AChE, was used in measurements of BuChE.

When 0.01 *mM* physostigmine was used in place of either of the other inhibitors, no reaction occurred in the CL of any of the 3 species, indicating the absence of physostigmine-resistant non-specific esterases. Allowance was made in each experiment for non-enzymic hydrolysis of substrate, and enzyme activity was expressed in nmoles of substrate hydrolysed per *mg* wet weight tissue per minute.

The slices taken for histochemistry were fixed for 4 hr in 10% formaldehyde in isotonic sodium sulphate at 4°C and kept overnight at 4°C in 20% ethanol. Sections were cut on a freezing microtome and after pretreatment with the appropriate inhibitor processed either for AChE (sheep, pig, cow) or for BuChE (cow) according to Lewis's (6) modification of Koelle's thiocholine technique (see 7). Control sections, incubated without substrate, ensured that the histochemical end-product was not confused with endogenous pigmentation.

RESULTS

Fig. 1 shows the mean AChE activity in corpora lutea of the sheep at different times after mating. Ringed figures indicate the number of CL analysed; all came from single animals,

except those for days 15 and 80 on each of which days , 2 animals were used. The CL analysed on day 143 came from a ewe which had given birth to 3 lambs a few hours previously.

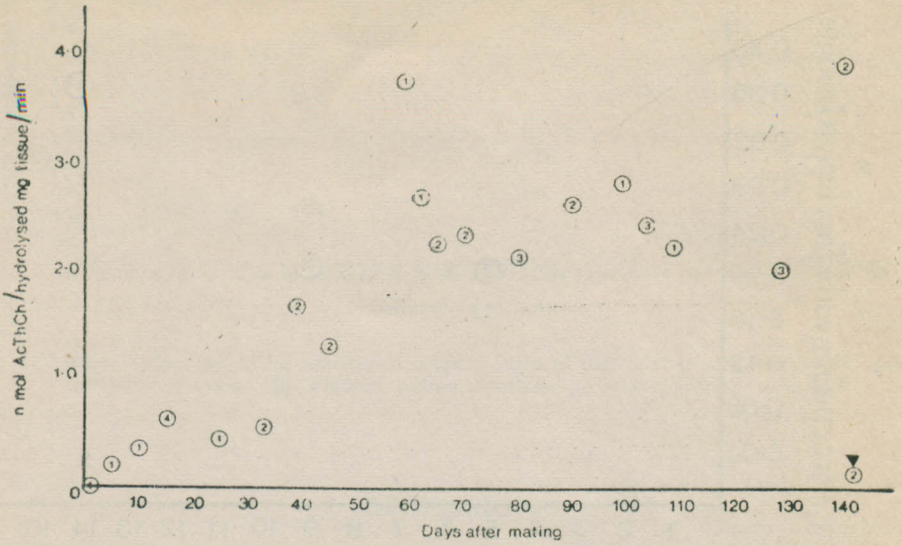

Fig. 1: Mean values for AChE activity in corpora lutea of pregnant sheep. Ringed figures indicate number analysed. ▼indicates animal examined post-partum.

The values for the individual corpora lutea from a single animal were generally in close agreement but occasionally they were discrepant. In such cases the sections processed histochemically invariably showed that while the intensity of the reaction in the luteal cells was of the same order in both corpora, that giving the lower analytical value contained larger amounts of non-reactive connective tissue. Statistically, there was a significant positive correlation between the time after mating and the AChE activity (Spearsman's $P \lessgtr +0.96$, $p < 0.01$).

AChE activity in the CL of sheep during the oestrous cycle is shown in Fig. 2. Values for individual animals are plotted separately and the ringed numbers indicate the number of corpora lutea present. One of the ewes scheduled to be used on the 14th day of the cycle ovulated on that day. The regressing CL of the cycle just ended showed only 10% of the activity in the CL from the other animal taken at the same interval after the previous oestrus. The histochemical appearance of the corpora from the two ewes was very different, the luteal cells in the CL of the sheep which had ovulated were ill-defined and stained only faintly.

Pigs: Results from the ovaries of the 12 pigs examined during pregnancy and of 2 pigs examined during the oestrous cycle are shown in Fig. 3. Although the series is limited the results suggest that, as in the sheep, AChE activity in the CL rises during pregnancy to levels exceeding those found in the CL of the oestrous cycle.

The rise in activity in the pregnant pig occurs between 12 and 15 days. This period coincides with the time at which the sow switches from the hormonal state of the oestrous cycle to the

hormonal state of pregnancy. More experiments are necessary to show whether the apparent decline after the early rise is genuine. Some evidence to support this possibility was provided by

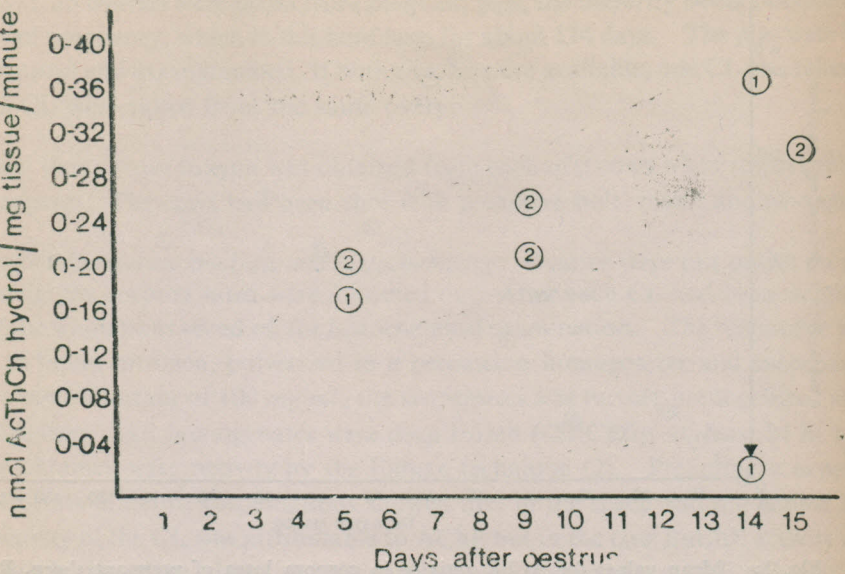

Fig. 2: Mean values for AChE activity in corpora lutea of sheep during the oestrous cycle. Ringed figures indicate number analysed. ▼indicates animal which had just ovulated.

2 histochemical experiments done on the 100th and 105th day of pregnancy. In both animals the intensity of staining in the CL was considerably weaker than that seen on the 16th day.

Cows: Results obtained from the cows were puzzling in that the histochemical and analytical findings were at variance. The histochemically treated sections of the CL from all 3 cows stained strongly for AChE and, unlike the sheep and pig CL, they also stained strongly for BuChE. Although the cow CL is particularly heavily pigmented it was clear from control sections that the enzymatically-produced staining could be easily distinguished from the pigment. The figures for AChE activity obtained spectrophotometrically were, in all 3 cows, somewhat lower than might be expected (on the basis of results in sheep and pigs) from the intensity of the histochemical reaction. Furthermore, although the histochemical reaction for BuChE appeared to be equal to, or even greater than that for AChE, the results of the quantitative analyses suggested that BuChE activity was extremely low, 10% or less of the AChE activity. In an attempt to explain this discrepancy, some alterations were made in the analytical method so that condttions with respect to pH and type of buffer paralleled more closely the conditions in the histochemical method. All such alterations resulted in even lower analytical values and the significance of the lack of parallelism between the two methods is unresolved. Moreover, because of this problem, the quantitative values of 1-2 *nmol/mg/min* for AChE activity obtained in all 3 cows cannot be regarded as reliable.

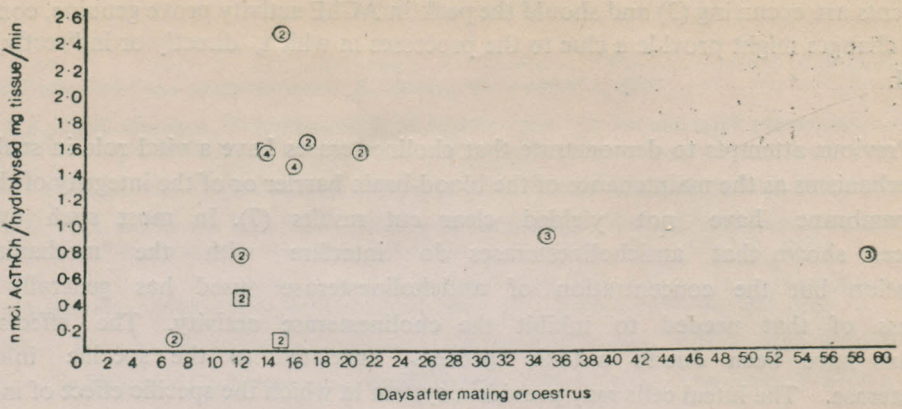

Fig. 3: Mean values for AChE activity in corpora lutea of pigs during pregnancy, O, and the oestrous cycle, ■. Figures within symbols indicate number analysed.

DISCUSSION

A point to be kept in mind when measuring cholinesterase activity in a tissue is whether the enzyme is associated with the tissue itself or with the blood it contains. In the present study attempts were made, initially, to estimate the amount of blood in each CL on the basis of the haemoglobin content of the homogenates. The method proved unreliable, however, apparently because of interference by luteal pigment. This meant that the proportion of the total activity which was attributable to blood cholinesterase could not be calculated but it is clear from the histochemically processed sections that the luteal cells in all 3 species do contain cholinesterase; furthermore, in the sheep and pig, changes in the intensity of the reaction in the cells during pregnancy paralleled the changes in the quantitative results. Since sheep and pig blood do not react histochemically for AChE (cf cat, 9) this implies that there is a specific increase in the AChE activity of the luteal cells themselves in addition to any rise in total AChE resulting from increased vascularity.

At present, the function of the cholinesterases in the corpus luteum is obscure. Moreover as has been pointed out previously (8,9) there is no evidence that non-neuronal cholinesterases in the ovary are necessary to its proper function. Although it seems unlikely that their synthesis is an uncontrolled side effect of other processes within the ovary this possibility cannot, as yet, be dismissed. In the sheep, the pattern of change in pregnancy did not parallel a recognized change in any other parameter such as progesterone production (1). The apparent coincidence, in the pig, of a rise in activity during the time of maternal recognition of pregnancy (3) requires confirmation from a larger series of animals. At this stage of pregnancy, a number of hormonal

adjustments are occurring (3) and should the peak in AChE activity prove genuine, consideration of these changes might provide a clue to the processes in which, directly or indirectly, AChE is involved.

Previous attempts to demonstrate that cholinesterases have a vital role in such non-nervous mechanisms as the maintenance of the blood-brain barrier or of the integrity of the erythrocyte membrane have not yielded clear cut results (7). In most such experiments it has been shown that anticholinesterases do interfere with the mechanism under investigation but the concentration of anticholinesterase used has generally been far in excess of that needed to inhibit the cholinesterase activity. The effects observed may thus have been due to a toxic process unrelated to the specific inhibition of cholinesterase. The luteal cells may provide a system in which the specific effect of inhibition of non-neuronal cholinesterase can be studied more precisely. I have previously reported (9) that the luteal cells of the pregnant rat and pregnant roe deer contain, respectively, BuChE and AChE, and have suggested that a comparison of the effects of specific inhibitors of BuChE and AChE on the synthetic activity in cultures of the cells from the 2 species might indicate whether the cholinesterases were involved in any synthetic process. The pig ovary, with its large number of corpora could be particularly useful for such a study. The cow CL, too, might provide interesting data since it appears to contain both types of cholinesterase but before any meaningful inhibitor studies are undertaken, it will be necessary to establish the optimal conditions for quantitative analysis in this species. Should the specific anticholinesterases prove to have no effect on the synthetic activity of the CL this would tend to support the possibility that the enzymes are non-functional and are induced as a secondary result of factors operating on or within the ovary.

Further studies on luteal cholinesterases, whether designed to discover the role of the enzymes in the ovary specifically, or in non-neuronal tissue in general, must thus take account of changes in steroidogenesis in the ovary and of changes in the hormones acting on it. In addition, it is necessary to consider species differences in the nature of the luteal enzyme. The recent interesting suggestion of Koelle *et al.* (4,5) that, in ganglia, BuChE is a precursor of AChE may be relevant in this context.

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