CHAPTER-5

A STUDY ON THE EFFECTS OF SIMULTANEOUS ADMINISTRATION OF 17β -ESTRADIOL AND PROGESTERONE ON CERTAIN METABOLIC PATTERNS OF SUBMANDIBULAR GLAND.

Though the immediately preceding chapter dealt with the influence of 17β-estradiol replacement on 48 H ovariectomized rats it is a common fact that under normal physiological conditions of estrous cyclicity other hormones also come into play, especially the progesterone. Hence, it is imperative that similar investigation should be carried out with regard to progesterone on the parameters of submandibular gland considered earlier. However, it is a known fact that various sensitive organs/tissues first come under principally the influence of estrogenic hormones and then the gestagenic ones, it was thought desirable to look into the influence of simultaneous administration of 17β-estradiol (E_2) and progesterone (P) administered together. This may enable one to see whether the latter acts in synergistic fashion or in an antagonistic manner with respect to different parameters.

Pertinent literature on estrogen-sensitivity of submandibular gland of rat and the effects of estrogens on submandibular gland, saliva and also other sensitive tissues has been cited previously. Here some other reports on either individual actions of natural or synthetic estrogen and progestagenic compounds or in combination could be referred to. Dahm *et al.* (1977) have reported on the influence of progesterone administration on certain enzymes of carbohydrate and lipid metabolism in the liver of rat and have opined that progesterone effects are compatible with action of insulin and also induce glycogenesis. *In vitro* studies of Ferguson and Bannon (1983) on progesterone metabolizing capacity of submandibular gland of female mice have demonstrated the role of 20α-hydroxysteroid dehydrogenase enzyme activity in the gland. According to Campos *et al.* (1984) administration of medroxyprogesterone acetate to female mice brings

about an increase in weight of submandibular gland and induces enlargement as well as masculinization of granular ducts. Further, they also stated such an influence was observable even in ovariectomized mice.

Liu (1968), working on submandibular gland of rat, have reported that synthetic gestagenic and progestagenic combination doses of Mesterol and Norethynodrel resulted in reduction in diameter and number of granular tubules. A very interesting finding about the individual and combined action of estrogen and progesterone on uterine tissue of ovariectomized rat has been reported by Rinard (1972). He took into consideration only the phosphorylase enzyme activity in the tissue and reported that administration of progesterone alone leads to some stimulation of this enzyme activity but that of estrogen was maximum, whereas the two hormones in combination bring about an intermediate response of the enzyme. White and Mudd (1975), taking into consideration the autoantigenicity induction and some morphological changes, have opined that progesterone induced autoantigenicity in submandibular gland of rat. Whereas the combination regime bring about only morphological changes of the gland. Magnusson *et al.*(1975) have reported that a combination oral dose of synthetic gestagen and progesterone leads to alterations in several salivary components of women. Rinard (1975) investigated the influence of estrogen, progesterone and catacholamines on rat uterine levels of c.AMP and glycogen phosphorylase of ovariectomized rats. His work has shown that estrogen alone strongly stimulated generation of c.AMP. It is, therefore, clear that estrogen and progesterone administered severally or in combination may affect different metabolic reactions either in synergism or in an antagonistic manner. The present chapter therefore, reports on the influence of simultaneous administration of 17β estradiol and progesterone on certain metabolites and enzyme activities of the submandibular gland of 48 H spayed rats.

MATERIAL AND METHODS

48 H ovariectomized females were injected with three different doses of 17β-estradiol i.e. 5 (CD-1), 10 (CD-2) and 15 (CD-3) μg simultaneously with 2 mg of progesterone in each case. Single intramuscular injection of hormonal mixture in 0.5 ml propylene glycol was administered to each animal. All the hormone replaced as well as control females were sacrificed after two H of injection. 48 H ovariectomized animals injected with 0.5 ml of propylene glycol served as controls. The results obtained with 48 H ovariectomized females and those treated with 0.5 ml vehicle did not show any noticeable difference hence, the latter readings were considered as redundant. All the parameters were estimated as per methods given in chapter-1.

RESULTS

The presently obtained results showed increase in glandular glycogen concentration at 2 H with CD-1 to CD-3, particularly with higher estrogen levels. Total phosphorylase activity was found to rise significantly with CD-1 and CD-3 whereas with CD-2 the same was brought almost to normal level (a corrective regime). On the other hand, the c.AMP-PDE activity decreased significantly with CD-1 and CD-3 , but it showed increased activity with CD-2. Total ATPase enzyme activity registered significant rise in activity with all the three doses, however, Na^{+}-K^{+} ATPase enzyme activity revealed reduction with all the three doses. SDH activity registered increasedactivity with CD-1, but exhibited a normalizing trend with CD-2 and CD-3.

A very significant hyperglycemic condition was found to be induced by CD-1 and CD-2 but CD-3 elicited a marked hypoglycemic response.

TABLE-5.1

Showing the influence of simultaneous administered fixed dose of 2 mg of progesterone with 5 (CD-1), 10 (CD-2) and 15 (CD-3) µg of 17β-estradiol (E_2) administration to 48 H ovariectomized females sacrificed after 2 H on various biochemical parameters of submandibular gland.

Parameters	Diestrous female normal	48 H OvX female	Doses administered		
			CD - 1	CD - 2	CD - 3
Glycogen mg/ 100 mg tissue	0.066 ± 0.006	0.121[a] ± 0.007	0.091[b] ± 0.005	0.167[a] ± 0.008	0.116[a] ± 0 001
Phosphorylase µmoles PO_4 released/ mg protein/ H	73.538 ± 02.026	71.304 ± 01.370	95.439[a] ± 01.613	68.042[c] ± 01.977	83.823[b] ± 02.240
c AMP-PDE µmoles PO_4 released/ mg protein/ H	4.844 ± 0.515	5.464 ± 0.203	2.315[a] ± 0 129	5.801[a] ± 0.232	2.823[b] ± 0.207
Total ATPase µmoles PO_4 released/ mg protein/ H	200.328 ± 012.00	225.619 ± 009.394	226.393[c] ± 000.129	268.843[a] ± 006.739	261.92[a] ± 03.769
Na^+-K^+ ATPase µmoles PO_4 released/ mg protein/ H	41.824 ± 03.175	14.859[a] ± 01.023	22.415[a] ± 01.696	25.927[a] ± 01.333	34.571[c] ± 01.006
SDH µg Formazan formed/ mg protein/ H	30.304 ± 01.726	47.784[a] ± 01 705	53.875[a] ± 00.905	33.291 ± 01.351	34.567[c] ± 01.416
Protein mg/ 100 mg tissue	24.467 ± 01.446	21.448[c] ± 00.894	22.647 ± 00.905	23.416 ± 00.583	27.096 ± 00.673
Plasma glucose mg/ 100 ml plasma	112.500 ± 002.012	100.500[a] ± 000.948	141 00[a] ± 03.000	155.00[a] ± 002.571	79.714[a] ± 03.636

Values are mean ± SE (n = 8)
a - P<0.0005; b - P<0.005; c - P<0.05
Levels of significance have been calculated with reference to diestrous values.

The CD-1 and CD-2 doses were found to maintain normal protein levels. Total protein concentration exhibited noticeable increase only with CD-3 regimen of hormone replacement.

DISCUSSION

It is a well recognized fact that generally the effects of progesterone require priming with or concurrent presence of estrogens for their full manifestation. These classes of ovarian hormones may act either synergistically(Demers and Jacobs, 1973; Ahmed-Sorovr and Bailey, 1980) or in antagonistic (Garrison *et al.*.1973; Bo, 1977; Tripathi and Krishnan, 1985; Carrington and Bailey, 1985; Ishihara *et al.*,1988) manner depending on animal species as well as tissues under consideration.

It is evident from the tabulated values for different parameters under study that simultaneous administration of progesterone acted clearly in an antagonistic manner in case of levels of glycogen and protein as well as SDH activity. On the other hand, in the case of total ATPase activity combination of P with E_2 was certainly synergistic in action. This was also true for only CD-1 in case of Na^+-K^+ ATPase but otherwise P acted in an antagonistic way. In case of rest of the parameters too, with merely the exception, of protein, progesterone influence was of antagonistic nature. Further, in most of the cases influence of CD-2 was more obvious. Hence, the following observations are made on the basis of these considerations. Foregoing recapitulations revealed that simultaneous administration of P favoured build up of glandular protein as well as glycogen concentration. Some of the factors that could be of complementary nature are normalization of SDH and lowering of Na^+-K^+ ATPase enzyme activities, which might reduce comparatively high catabolic activity of submandibular gland that was apparently induced by E_2 alone.

However, there were a few paradoxical influences which need explanation. Eventhough values of phosphorylase were higher (with CD-1 and CD-3) and those of glandular glycogen too, were high, the variations of c.AMP-PDE activity seemingly hindered actual manifestation of glycogen breakdown by phosphorylase (with CD-1 and CD-3) and probably lowered Na^+-K^+ ATPase activity complemented this overall metabolic state. Further, it was a generally observed fact that normally reduction of c.AMP-PDE activity leading to increase availability of intracellular c.AMP for usual stimulatory action on phosphorylase to take about more than an hour to get manifested in alterations of glycogen concentration. Even total ATPase activity was also noticeably moderated by combination doses. Another aspect concerned the high levels of plasma glucose (CD-1 and CD-2) The site of such a hyperglycemic influence is not this gland but the liver of the animal, as has been reported by Wagh (1994). From this it is evident that 17β-estradiol plus progesterone administration influences the liver in a synergistic manner as far as release of glucose into blood by the liver is concerned.

By way of summerizing these observations it could be said that administration of estradiol alone that induced an overall catabolic pattern of metabolism in submandibular gland of 48 H ovariectomized rats, was reversed to a considerable extent by simultaneous administration of fixed dose of 2 mg progesterone with 5, 10 and 15 μg of 17β-estradiol. Further, it is also seen that CD-2 was more effective particularly at the 2 H interval in more or less restoring the functional state of submandibular gland of ovariectomized rats. One additional point to be noted is that variation of glycemic level does not depend on the status of either the glandular glycogen or phosphorylase, but are related to synergistic action of combined hormonal doses on the liver.