INFLUENCE OF PREGNENOLONE-16α-CARBONITRILE ON SPERMATOGENESIS AND ON THE SPERMATOGENIC ACTIVITY OF PREGNENOLONE

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SUMMARY

In hypophysectomized rats, treatment with pregnenolone (pregn-5-en-3β-ol-20-one) for 14 days prevented testicular atrophy, maintained spermatogenesis but did not counteract Leydig cell atrophy. Spermatogenic activity was abolished when a 16α-nitrile group was attached to the pregnenolone molecule, but pregnenolone-16α-carbonitrile (PCN) did not interfere with the spermatogenic effect of pregnenolone. These results are discussed in view of the fact that PCN inhibits intoxication with excess amounts of many steroids (e.g. anaesthesia due to heavy overdosage with progesterone or hydroxydione), whereas steroid hormones, administered at doses necessary to maintain physiological functions, are virtually resistant to this type of catatoxic action, which usually takes the form of enhanced metabolic degradation and/or substrate excretion.

INTRODUCTION

Walsh, Cuyler & McCullagh (1934) and Nelson & Gallagher (1936) were the first to show that male sex hormones extracted from urine can maintain spermatogenesis in adult hypophysectomized rats. Cutuly, McCullagh & Cutuly (1937) obtained similar results with synthetic androgens. However, it soon appeared that the capacity of these substances to maintain spermatogenesis in hypophysectomized rats was independent of their androgenic activity (Nelson & Merckel, 1937); steroids devoid of such properties (e.g. progesterone and pregnenolone) were also found to inhibit testicular atrophy (Nelson, 1936; Selye & Friedman, 1941; Albert & Selye, 1942). Pregnenolone and its esters proved to be the purest spermatogenic steroids, being practically devoid of all other hormonal effects (Selye, 1942; Leathem & Breant, 1943; Ruzicka & Prelog, 1943; Masson, 1944, 1945, 1946). Recently, this activity was ascribed to some of its androstanol metabolites although, as mentioned above, pregnenolone itself could not give rise to the production of very potent androgens (Kim & Straw, 1971).

Earlier investigations in our laboratory (Selye, 1971) have shown that certain steroids protect the rat against various intoxications. In most cases, these catatoxic

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(from the Greek 'kata' = down, against) steroids enhance the transformation and/or excretion of the toxicants (Solymoss, Varga & Krajny, 1970, 1971) or steroid hormones, including progesterone (Zsigmond & Solymoss, 1971), through the induction of drug-metabolizing enzyme activity in liver microsomes (Solymoss, Werring-loer & Toth, 1971). Systematic studies (Selye, 1971) have demonstrated that, among more than 1200 steroids tested, pregnenolone-16a-carbonitrile (PCN) is one of the most potent catatoxic compounds in that it exerts the greatest prophylactic effect against numerous toxic agents in vivo. Because of its close structural relationship to pregnenolone, the question arose whether PCN has spermatogenic activity in hypophysectomized rats and whether, owing to its catatoxic properties, PCN can influence the spermatogenic activity of pregnenolone.

MATERIALS AND METHODS

Forty-nine adult male Sprague-Dawley rats (Canadian Breeding Farms & Laboratories Ltd., St. Constant, Quebec) weighing 110–120 g and maintained on a commercially available diet (Purina Laboratory Chow) and tap water ad libitum, were divided into six groups, of which group 1 served as controls. Groups 2–6 were hypophysectomized by the parapharyngeal route (Selye, 1949) on the 1st day of the experiment. For the next 14 days, groups 3–6 received 2 mg of pregnenolone (Schering) in 0.2 ml peanut oil, and/or 2 or 10 mg of PCN [3β-hydroxy-20-oxo-5-pregnene-16α-carbonitrile (Upjohn)] in 0.2 or 0.5 ml peanut oil, respectively (Table 1). Both steroids were injected once daily s.c. To obtain the best catatoxic effect, group 6 was treated with PCN for 3 days before hypophysectomy and with

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Final body wt (g)</th>
<th>Organ weights (g/100 g body wt)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Testes</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>10</td>
<td>217 ± 4</td>
<td>0.075 ± 0.032***</td>
</tr>
<tr>
<td>2</td>
<td>Hypophysectomy</td>
<td>7</td>
<td>106 ± 3</td>
<td>0.053 ± 0.055</td>
</tr>
<tr>
<td>3</td>
<td>Hypophysectomy + pregnenolone (2 mg)</td>
<td>10</td>
<td>112 ± 2</td>
<td>0.141 ± 0.046***</td>
</tr>
<tr>
<td>4</td>
<td>Hypophysectomy + PCN (2 mg)</td>
<td>7</td>
<td>105 ± 4</td>
<td>0.364 ± 0.035 NS</td>
</tr>
<tr>
<td>5</td>
<td>Hypophysectomy + PCN (10 mg)</td>
<td>8</td>
<td>107 ± 5</td>
<td>0.535 ± 0.077 NS</td>
</tr>
<tr>
<td>6</td>
<td>Hypophysectomy + PCN (10 mg) + pregnenolone (2 mg)</td>
<td>7</td>
<td>127 ± 4</td>
<td>0.161 ± 0.055***</td>
</tr>
</tbody>
</table>

* Significance of difference from group 2 (statistically calculated by analysis of covariance on the 15th day): *** P < 0.001; NS = not significant (P > 0.05).

The means of groups 1 and 2 are not significantly different. This is due to a marked inequality in the covariance of these groups. The discrepancy reflects an association of a smaller covariance with a smaller mean.
PCN plus pregnenolone for 14 days after the operation. Autopsies were performed on the 15th day.

The completeness of hypophysectomy was verified macroscopically, and the testes, seminal vesicles and preputial glands were removed and weighed (Table 1). [Organ weight is expressed per 100 g of body weight (mean ± s.d.).] For statistical evaluation, analysis of covariance was used; organ weight regressed on body weight (Snedecor & Cochran, 1967). For histological examination, these organs were fixed in a Susa’s solution saturated with picric acid, and stained with haematoxylin–phloxine or by the periodic acid–Schiff technique.

RESULTS

The testes of the controls (group 1) contained normal seminiferous tubules (Plate, fig. 1). The weight of the testes and secondary sex glands was greatly decreased 14 days after hypophysectomy (group 2); the seminiferous tubules were devoid of spermatocytes, and the interstitial tissue was atrophied (Plate, fig. 2).

In the pregnenolone-treated rats (group 3), the weight of the testes was normal, whereas that of the seminal vesicles was below normal. Histologically, large seminiferous tubules and numerous spermatocytes were visible (Plate, fig. 3). However, spermatogenesis was not completely restored because desquamous cells were present in the seminal vesicles of these animals.

PCN, at the dose of 2 or 10 mg (groups 4 and 5), did not inhibit testicular atrophy caused by hypophysectomy. The histological features were similar to those of the hypophysectomized animals (Plate, fig. 4). PCN did not interfere with the maintenance of normal testicular weight by pregnenolone (group 6). Light microscopically, spermatogenic activity was evident in these animals (Plate, fig. 5).

DISCUSSION

Our results indicate that treatment with pregnenolone for 14 days after hypophysectomy maintains spermatogenesis without restoring the endocrine function of the testis, whereas PCN has no effect on either of these two parameters. Thus, the introduction of a 16α-carbonitrile group significantly modifies the pharmacological activity of pregnenolone by transforming it into a highly active catatotoxic compound, devoid of spermatogenic potency. This is of special interest in view of the fact that, whereas PCN is one of the most potent catatotoxic steroids known to date (Selye, 1971), pregnenolone itself has no microsomal enzyme-inducing properties (Solymoss et al. 1971).

It has been claimed that testicular weight and spermatogenesis are maintained by pregnenolone indirectly through androgenic metabolites produced in situ within the testis (Kahnt, Neher, Schmid & Wettstein, 1961; Kim & Straw, 1971). The spermatogenic effect of progesterone might be explained in a similar manner (Lacassagne, 1971). The fact that cyproterone, a potent antiandrogen, inhibits the effect of testosterone, not only upon the prostate and seminal vesicles but also upon the seminal epithelium of the hypophysectomized rat (Neumann & von Berswordt-Wallrabe, 1966), was interpreted in the same way.
This view was supported by the observation that the spermatogenic action of progesterone is also inhibited by cyproterone in hypophysectomized rats. From this latter finding it was concluded 'that the former hypothesis that spermatogenic potencies are entirely independent pharmacological activities of steroids, is no longer tenable, at least for progesterone, as tested in this study' (Neumann & von Berswordt-Wallrabe, 1966). Yet, both pregnenolone and progesterone are devoid of any significant androgenic activity in castrated or immature rats (Selye, 1943) and, besides being a potent antiandrogen, cyproterone has considerable catatotoxic activity against numerous substrates, including progesterone (Selye, 1971); hence, its antispermatogenic effect is not necessarily dependent upon its antiandrogenic activity. In any case, pregnenolone is a much more potent spermatogenic steroid than would correspond to its minimal, if it exists at all, androgenic activity; hence, the former effect cannot be directly dependent upon the latter.

Even if it should become possible to show that pregnenolone is transformed into highly potent androgens in the testis and that these components are responsible for the maintenance of the seminal epithelium, this would only help to elucidate the underlying mechanism of its action. It would show that, although not androgenic in itself, pregnenolone has the singular property of giving rise to spermatogenic androgens locally in the target organs, in this instance, the testis. If so, we would have to conclude that although certain compounds (e.g. progesterone and pregnenolone) have no systemic androgenic effect of their own, they can give rise to the production of androgens under certain circumstances in the presence of appropriate enzyme systems. These androstanes might be intermediates in the exertion of spermatogenic activity, just as the specific effects of progesterone, testosterone and oestradiol are independent of each other and can yet be produced from cholesterol or pregnenolone by appropriate enzyme systems localized in certain target tissues.

The present investigation also shows that PCN does not interfere with the maintenance of spermatogenesis in hypophysectomized, pregnenolone-treated rats; that is, under our experimental conditions it does not exert catatotoxic activity against this particular effect of pregnenolone. On the other hand, the anaesthetic action of progesterone in high doses is completely blocked by PCN, and this inhibition is associated with decreased levels of progesterone and its anaesthetic metabolites in blood and brain (Zsigmond & Solymoss, 1971).

The presence of the hypophysis is certainly not indispensable for the effect of PCN since the latter accelerates the transformation of many drugs and steroid hormones in vivo, and causes proliferation of the hepatic smooth-surfaced endoplasmic reticulum even in hypophysectomized rats (Garg, Szabo, Khandekar & Kovacs, 1971; Szabo, Kovacs, Garg, Khandekar & Selye, 1971). Perhaps the spermatogenic activity of pregnenolone or that of its active metabolites, being localized in the testis, is protected against enzymatic destruction in the liver. We have established that PCN does not interrupt pregnancy in intact rats (Selye, Taché & Szabo, 1971) nor in rats after ovariectomy and maintenance of gestation with progesterone and oestrone (Y. Taché, unpublished observations). Since the development of pregnancy is impossible without ovarian hormones, the operation of some mechanism which protects the ovarian hormones against degradation by catatotoxic steroids at certain crucial points of their metabolism may again be suspected. Thus, hormones ad-
ministered for the maintenance of physiological functions do not seem to be sensitive to the catatonic action of PCN. An important part of the injected hormones could be bound to proteins or metabolized by enzymes other than those induced by PCN. Biochemical studies are needed to elucidate these possibilities.

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REFERENCES


DESCRIPTION OF PLATE

Seminiferous tubules of rats. Magnification: × 120.

Fig. 1. Seminiferous tubules of normal rat. Periodic acid Schiff (PAS).

Fig. 2. Atrophy of seminiferous tubules and interstitial tissue 15 days after hypophysectomy. No spermatocytes are visible. PAS.

Fig. 3. Hypophysectomized rat treated with pregnenolone for 14 days. The spermatocytes are normal while the interstitial cells are atrophied. Haematoxylin–phloxine.

Fig. 4. Hypophysectomized rat given pregnenolone-16α-carbonitrile (PCN) for 14 days. Note the atrophy of the tubules and the absence of spermatocytes. PAS.

Fig. 5. Hypophysectomized rat pretreated with PCN and post-treated with PCN plus pregnenolone from day of hypophysectomy. Spermatogenic activity is normal. The interstitial cells are atrophied. PAS.