EFFECT OF CONSTANT LIGHT AND ANDROGEN-STERILIZATION ON THE AMINE TURNOVER OF THE TUBERO-INFUNDIBULAR DOPAMINE NEURONS: BLOCKADE OF CYCLIC ACTIVITY AND INDUCTION OF A PERSISTENT HIGH DOPAMINE TURNOVER IN THE MEDIAN EMINENCE

By

K. Fuxe, T. Hökfelt and O. Nilsson

ABSTRACT

The dopamine (DA) turnover in the median eminence and in the neostriatum in the rat has been studied during the normal ovarian cycle, in rats exposed to constant light and in androgen-sterilized rats treated with the tyrosine hydroxylase inhibitor α-methyl-tyrosine-methylester. The rate of depletion of DA provides an estimation of the turnover and this can be evaluated semi-quantitatively by means of the highly sensitive and specific histochemical fluorescence method of Falck and Hillarp for the demonstration of catecholamines (CA).

The results reveal cyclic turnover changes in the tubero-infundibular DA neurons during the ovarian cycle but not in the neostriatal DA neurons. The turnover is decreased during pro-oestrus and early oestrus. In the persistent oestrous rats, whether induced by constant light or by neonatal steroid treatment, no cyclic changes in DA turnover in the median eminence occurred. The turnover remained at a constant high level in these rats, similar to that found in dioestrus of normal cycling rats. The high oestrogen secretion in persistent oestrous rats was probably responsible for the high DA turnover, since in these rats castration caused a marked

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reduction in the DA turnover. Testosterone and oestrogen caused a dose-dependent increase in DA turnover in the median eminence in androgen-sterilized castrated rats. The DA neurons of the androgen-sterilized castrated rats were found to be somewhat less sensitive to treatment with oestrogen than those of normal castrated rats. The present results support the view that the tubero-infundibular DA neurons participate in the inhibition of the cyclic release of LHRF and FSHRF from the median eminence and that the inhibitory feed-back action of oestrogen and testosterone is partly mediated via an increase in DA turnover and release in the median eminence, resulting in inhibition of LHRF and FSHRF release.

In previous papers (Fuxe et al. 1967, 1969b) it has been reported that the activity of the tubero-infundibular DA neurons is markedly increased during pregnancy, lactation and pseudo-pregnancy, i.e. conditions in which there is a low FSH and LH secretion and blockade of ovulation. Furthermore, it has been briefly reported (Fuxe et al. 1967) that the tubero-infundibular DA neurons undergo cyclic activity changes during the oestrous cycle with low activity during pro-oestrus and early oestrus, suggesting that at the time of LHRF release the activity of the system is low. Subsequently, a detailed study revealed a decrease in DA activity in the median eminence already in early pro-oestrus before the critical period. The release of LH in the afternoon of pro-oestrus was indicated in the same group of rats as a fall in pituitary LH content (Ahrén et al. 1971).

All these findings suggest that the tubero-infundibular DA neurons may be involved in the control of gonadotrophin secretion from the anterior pituitary. In view of the turnover changes observed it was postulated that the DA neurons in the median eminence act partly by inhibiting LHRF secretion from the median eminence. In order to study this hypothesis, other conditions involving blockade of ovulation have been studied, i.e. the persistent oestrous syndrome induced by constant light or by androgen-sterilization. Furthermore, castration and treatment with sex hormones have been performed in androgen-sterilized rats.

MATERIAL AND METHODS

Animals
Female Sprague-Dawley rats b. wt. 150–180 g) were used. The animals were housed at a room temperature of +24°C ± 1°C. They were kept on a semisynthetic diet and drank ad libitum. They had a light cycle lasting 14 hours, starting at 6 a.m. and ending at 8 p.m. The stage of the ovarian cycle was determined by means of daily vaginal smears, an additional smear being taken at the time of killing and also by means of vaginal sections. On the day of pro-oestrus the uterus was always found
to be distended. Only animals with regular 4 day cycles were included in the present material.

Androgen-sterilization was performed by injecting 1.25 mg of testosterone propionate into 2 day old female rats. The control rats received castor oil injections. The androgen-sterilized rats were used in the present experiments when 2 months of age. These rats showed persistent vaginal cornification, and the ovaries were polyfollicular and contained no corpora lutea.

The rats exposed to constant light (2–3 months) also showed persistent vaginal cornification and also had polyfollicular ovaries.

Castration of the androgen-sterilized rats and of the rats exposed to constant light was performed 3 weeks before killing. A large number of androgen-sterilized castrated rats were treated either with β-oestradiol benzoate or with testosterone propionate as described in Table 2. The injections were performed intramuscularly, and castrated controls received the solvent castor oil in corresponding amounts.

Fluorescence histochemistry

The amino turnover in the DA nerve terminals of the median eminence was estimated with the help of the tyrosine hydroxylase inhibitor, α-methyl-tyrosine-methyl-ester-hydrochloride (H44/68; 250 mg/kg, ip, 4 hours before killing) (see Andén et al. 1969). Thus, the rate of decrease of the amine stores after amine synthesis inhibition is a measure of turnover, the release of CA no longer being compensated for by synthesis. The inhibitor was given from 8–12 a.m. The histochemical method used was the histochemical fluorescence method of Falck and Hillarp for determining the cellular localization of catecholamines (Falck et al. 1962; Hillarp et al. 1966; Corrodi & Jonsson 1967).

It is possible to perform a semi-quantitative estimation of fluorescence intensity in the monoamine nerve terminals. Thus, it is known that a change in fluorescence intensity represents a change in the amine levels (Olson et al. 1968; Jonsson 1969). In contrast to the peripheral adrenergic neurons, the fluorescence concentration relationship seems to be linear in the cortical NA neurons and the DA neurons up to normal levels, thus allowing of good estimations of both the decrease and increase in intensity (Lidbrink & Jonsson, unpublished data). The evaluations were made on coded slides and by two investigators independently of one another. The following semi-quantitative estimations were made: 0 = no fluorescence; 1/2+ = very weak fluorescence; 1+ = weak fluorescence 2+ = moderate fluorescence; 3+ = strong fluorescence. Half a plus was added to the lower grade, if it could not be decided whether the fluorescence belonged to one grade or the other. The value from each animal represents the means of 6–8 estimations. It should be noted that the pluses are not quantitatively related. In order to test the specificity of the changes in the median eminence, the DA turnover in the neostriatum was also studied in all the animals in the present study.

RESULTS

Studies on DA levels in the median eminence

In none of the endocrine conditions studied (5–10 rats per experimental group) were there any definite changes in the fluorescence intensity of the DA nerve terminals as compared with normal cycling controls.
Ovarian cycle (Table 1)

In contrast to the normal male rats, normal female rats showed cyclic changes in amine turnover in the median eminence. Thus, the system was inactive in pro-oestrus (Fig. 1) and early oestrus, and at these stages of the cycle the system had an activity similar to that found in normal male rats. In the remainder of the cycle from late oestrus up to late dioestrus (Fig. 2), the system was active showing only a low fluorescence intensity in the DA terminals following treatment with H44/68. In all the rats studied there were no significant changes in the degree of disappearance of DA fluorescence in the neostriatum during the ovarian cycle.

Rats exposed to constant light (Table 1)

These rats did not exhibit any cyclic activity. Thus, they had a constant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fluorescence intensity after H 44/68 treatment</th>
<th>Effect compared with H 44/68 treated female rats in dioestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal female rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>metoestrus</td>
<td>(\frac{1}{2}+ (2)) 1+ (5) 2+ (1)</td>
<td>none</td>
</tr>
<tr>
<td>dioestrus</td>
<td>(\frac{1}{2}+ (1)) 1+ (6)</td>
<td></td>
</tr>
<tr>
<td>pro-oestrus</td>
<td>1+ (1) 2+ (5) 2(\frac{1}{2}+ (2))</td>
<td>retardation</td>
</tr>
<tr>
<td>early oestrus</td>
<td>2+ (4)</td>
<td>retardation</td>
</tr>
<tr>
<td>late oestrus</td>
<td>(\frac{1}{2}+ (2)) 1+ (6)</td>
<td>none</td>
</tr>
<tr>
<td>normal male rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>constant light 2 months</td>
<td>(\frac{1}{2}+ (4)) 1+ (4)</td>
<td>no cyclic activity</td>
</tr>
<tr>
<td>3 months</td>
<td>(\frac{1}{2}+ (4)) 1+ (4)</td>
<td>no cyclic activity</td>
</tr>
<tr>
<td>androgen-sterilized rats</td>
<td>(\frac{1}{2}+ (9)) 1+ (6)</td>
<td>no cyclic activity slight acceleration</td>
</tr>
</tbody>
</table>

Androgen-sterilization was performed by the injection of 1.25 mg of testosterone propionate on day 2. H 44/68 was given ip in a dose of 250 mg/kg 4 hours before sacrifice. A semiquantitative estimation of fluorescence intensity was made on coded slides by two investigators independently. 0 = no fluorescence; \(\frac{1}{2}+ = \) very weak fluorescence; 1+ = weak fluorescence; 2+ = moderate fluorescence; 3+ = strong fluorescence. Half a plus was added to the lower grade, if it could not be decided if the fluorescence belonged to one grade or the other. The value from each animal represents the means of 6–8 estimations. Observe that the pluses are not quantitatively related. Number of rats in brackets.
Fig. 1.
Naturally cycling rat in pro-oestrus. H.1168 treatment as described in text. A moderate fluorescence intensity is observed in the outer layer of the median eminence (2 ± in Table 1. × 150).

Fig. 2.
Naturally cycling rat in dioestrus. H.1168 treatment as described in text. A low fluorescence intensity is observed in the external layer of the median eminence (1 ± in Table 1. × 150).
Fig. 3
Female rat exposed to constant light for 2 months. H 44/68 treatment as described in text. A weak fluorescence intensity is observed in the external layer of the median eminence (1+ in Table 1). X 150.

Fig. 4
Androgen-sterilized rat. H 44/68 treatment as described in text. A very weak fluorescence intensity is observed in the external layer of the median eminence (1+ in Table 1). X 150.
high rate of disappearance in DA fluorescence in the median eminence similar to that found in normal female rats in dioestrus (Fig. 3).

**Androgen-sterilized rats (Table 1)**

The results were similar to those obtained in the constant light rats. Thus, there were no cyclic changes in the rate of disappearance of fluorescence from the DA nerve terminals of the median eminence. Instead a constant high rate of disappearance of fluorescence was observed similar or slightly higher than that found in dioestrus (Fig. 4).

**Castration**

**Normal female rats (Table 2).** – After castration no cyclic changes were observed in the median eminence. The rate of fluorescence disappearance was low and similar to that found in normal male rats.

**Rats exposed to constant light (Table 2).** – Castration abolished the constant high rate of disappearance of fluorescence found after H44/68 treatment. Instead a low rate of disappearance was now observed similar to that found in castrated female rats and in normal male rats. Castration did not influence the rate of fluorescence disappearance from the neostriatum.

**Androgen-sterilized rats (Table 2).** – Castration blocked the high rate of disappearance of fluorescence found in these rats after H44/68 treatment. Instead a very low rate of disappearance of DA fluorescence in the median eminence was observed which was even lower than that found in normal castrated female rats (Fig. 5). Castration did not induce any certain changes in the rate of fluorescence disappearance from the DA nerve terminals of the neostriatum.

**Effect of castration and treatment with sex hormones**

**Normal rats (Table 2).** – As already reported in a previous paper (Fuxe et al. 1969a) oestrogen was found to be capable of increasing the disappearance of fluorescence in the doses tested (down to 0.3 μg/rat). Testosterone was active in the doses tested (down to 0.3 mg/rat).

**Androgen-sterilized rats (Table 2).** – As in normal castrated female rats oestrogen in high doses was capable of inducing a marked acceleration of fluorescence disappearance in androgen-sterilized rats (Fig. 6). However, in lower doses starting at 3 μg/rat oestrogen was less effective and not effective at all in the lowest dose. Testosterone, on the other hand, had a similar potency in both low and high doses as observed in castrated female rats. Thus, the dose response curve for testosterone was similar in both androgen-sterilized castrated rats and in normal castrated rats.
Table 2.
Effect of castration and treatment with sex hormones on the H 44/68 induced fluorescence disappearance from the median eminence DA nerve terminals in androgen-sterilized rats, normal female rats and rats exposed to constant light.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fluorescence intensity after H 44/68 treatment</th>
<th>Effect compared with H 44/68 treated castrated rats in the various groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>constant light (2 months)</td>
<td>½± (6) 1± (5)</td>
<td>moderate to marked acceleration</td>
</tr>
<tr>
<td>castration + constant light</td>
<td>½± (4) 1± (3)</td>
<td>moderate to marked acceleration</td>
</tr>
<tr>
<td>androgen-sterilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>castration + androgen-sterilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;      &quot; + oestrogen</td>
<td>10 µg X 3 0 (4) ½± (4) 1± (2)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>5 µg X 3 0 (1) ½± (5) 1± (1)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>1 µg X 3</td>
<td>weak acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>0.1 µg X 3</td>
<td>none</td>
</tr>
<tr>
<td>&quot;      &quot; castor oil</td>
<td>5 mg X 3 0 (4) ½± (4)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>1 mg X 3</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>0.1 mg X 3</td>
<td>moderate acceleration</td>
</tr>
<tr>
<td>castration (normal female rats)</td>
<td>½± (2) 2± (8)</td>
<td></td>
</tr>
<tr>
<td>castration (normal female rats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;      &quot; + oestrogen</td>
<td>5 µg X 5 0 (3) ½± (4)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>1 µg X 3 0 (1) ½± (6) 1± (2) 1½± (2)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>0.1 µg X 3</td>
<td>moderate acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; testosterone</td>
<td>5 mg X 3 0 (3) ½± (3)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>1 mg X 3 0 (1) ½± (4) 1± (1)</td>
<td>marked acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; &quot;</td>
<td>0.1 mg X 3</td>
<td>moderate acceleration</td>
</tr>
<tr>
<td>&quot;      &quot; castor oil</td>
<td></td>
<td>none</td>
</tr>
</tbody>
</table>

Castration was performed 3-4 weeks before sacrifice. All injections were made im in castor oil. The injection schedule was always 3 injections at 24 h intervals. The H 44/68 treatment was performed as described in the text of Table 1. For further details see Text to Table 1. The acceleration of the H 44/68 induced fluorescence disappearance was considered as marked when most of the rats had a 1½±-2 plus change in intensity as moderate when most of the rats had a 1 plus change; as weak when most of the rats had only a half plus change.
Fig. 5.
Androgen-sterilized castrated rat. For further details see text. H 44/68 treatment as described in text. A fairly strong fluorescence intensity is observed in the outer layer of the median eminence (2V2 in Table 2). X 150.

Fig. 6.
Androgen-sterilized castrated rat treated with oestrogen 5 mg x 3; for further details see text. H 44/68 treatment as described in text. A very weak fluorescence intensity is observed in the outer layer of the median eminence (14A in Table 2). X 150.
DISCUSSION

The results of the present paper further underline the view (see Fuxe et al. 1967; Ahrén et al. 1971) that the tubero-infundibular DA neurons but not the neostriatal DA neurons undergo marked changes in amine turnover during the ovarian cycle and in addition show that this cyclic activity is abolished by castration, exposure to constant light or by androgen-sterilization. Furthermore, the results show that testosterone and oestrogen can produce a marked increase in amino turnover in the tubero-infundibular DA neurons not only in normal castrated rats, but also in androgen-sterilized castrated rats, although the sensitivity to oestrogen is somewhat less after androgen-sterilization as compared with normal rats. Since the DA turnover in the median eminence was low in pro-oestrus and early oestrus and since the DA turnover was high in conditions with blocked ovulation (constant light, androgen-sterilization), the present results support our hypothesis that the DA neurons act inter alia by inhibiting the cyclic release of LHRF from the median eminence. Such an inhibitory action of DA released locally in the median eminence would agree with the findings of a decreased turnover in pro-oestrus when LHRF is released to induce ovulation and the relatively high release of DA in conditions involving blocked ovulation.

The amine synthesis inhibition model used for studying amine turnover is a reliable method (see Andén et al. 1969; Fuxe et al. 1970). However, it is important that the method is used properly so that any possible sources of error can be avoided or be excluded. Since in the present study the turnover changes observed were only found in the median eminence, it can be excluded that the changes in the rate of depletion of amines were due to a changed metabolism of the inhibitor in the various experimental endocrine conditions studied. Furthermore, it is important to ensure that the degree of inhibition of the enzyme is similar in the control rats and in the experimental rats. This is mainly of importance when retardation of turnover is observed, since after the high doses of the inhibitor used, the degree of inhibition is usually maximal and no further inhibition of the enzyme can occur even if the doses of the inhibitor are markedly increased (Andén 1967). In a recent study we have therefore compared the degree of inhibition after treatment with H44/68 in normal, castrated and hypophysectomized male rats. The same marked degree of inhibition was found in all three types of animals, i.e. an inhibition of about 80–85% of tyrosine-hydroxylase. This study clearly suggests that turnover changes observed in endocrine conditions as compared with normal conditions are also due to changes in amine turnover and not due to changes in the degree of inhibition of the enzyme (Jonsson, Fuxe & Hökfelt, in preparation).

Since in a recent study (Fuxe et al., in press) it has not been possible to obtain any in vivo effects of oestrogen and testosterone on the uptake and accumula-
tion and retention of CA in central CA neurons, it seems likely that the turn-
over changes observed after treatment with sex hormones in various endocrine
conditions are mainly due to changes in the nervous impulse flow. Thus, it is
known that the amine turnover is highly dependent on the nervous impulse
flow (see Andén et al. 1966, 1967). A high impulse flow results in a high
turnover and a high release of the amine and vice versa when a low nervous
impulse flow is present. The changes in nervous impulse flow that probably
occur in the tubero-infundibular DA neurons following treatment with sex
hormones can be induced either by a direct action of the hormones on the DA
cell bodies themselves or indirectly by other neuron systems.

It is well known that persistent oestrous syndromes such as those studied in
the present paper show signs of considerable oestrogen production due to a
high tonic secretion of FSH and LH (see Lawton & Schwarz 1967; Negro-Vilar
et al. 1968), but show blockade of ovulation (Wurtman 1967; Barraclough
1967). The ovaries are polyfollicular and lack luteinization. The vaginal smears
show signs of oest us with cornification. The main lesion in these rats therefore
seems to be a blockade of the ovulation discharge of LHRF from the median
ingence. It is generally believed that this is due to a change in the properties
of the trigger centre in the preoptic area (see Reichlin 1965; Guillemin &
Rosenberg 1956; Deuben & Meites 1964; Bradshaw & Critchlow 1966; Gorski
1966) possibly partly induced by a decrease in the uptake of oestrogen in the
case of androgen-sterilized rats due to a blockade of oestrogen receptor sites
in this area (see Flerkó et al. 1969; McEwen & Pfaff 1970) and in the case of
the rats exposed to constant light probably due to the marked changes in
afferent impulses to this area. The constant relatively high release of DA in
the median eminence of androgen-sterilized rats and in constant light rats is
therefore probably not the primary cause of the blockade of ovulation found
in these rats. However, this DA release can contribute to the inhibition of the
LHRF discharge found in these states. Furthermore, the results indicate that
the cyclic behaviour of the DA neurons is induced via changes in the nervous
impulse flow in the trigger centre of the preoptic area. It may be that when
this centre is not working properly the inactivation of the DA neurons will no
longer occur during pro-oestrus.

It has previously been postulated that the DA neurons participate in me-
diating the inhibitory feed-back of oestrogen and testosterone on LH and FSH
secretion (Fuxe et al. 1969a). This view is further supported by the present
results, and the high oestrogen secretion in androgen-sterilized rats and in
constants light rats is probably mainly responsible for the constant high activity
in the tubero-infundibular DA neurons. This is also directly demonstrated by
the marked reduction of activity found in these rats after castration. Schiavi
(1969) has also recently demonstrated a marked increase in LH and FSH
pituitary and serum levels in testosterone sterilized rats after castration. This
gives additional support to our view that the DA released in the median eminence also mediates the negative feed-back of sex hormones on gonadotrophin secretion in androgen-sterilized rats. McCann & Ramirez (1964) have also reported signs of increased FSH and LH secretion in castrated constant light treated rats, which indicates that the same also holds true for the sex hormone feed-back in constant light rats. Thus, the negative feed-back action of sex hormones acts in androgen-sterilized rats and in constant light rats.

It is known that rats exposed to a standardized light-night schedule show a marked release of LHRF and FSHRF during a short period of about 2 h during pro-oestrus resulting in ovulation (see Gay et al. 1970). Since the inactivation of the DA neurons during the oestrous cycle occurs during a much longer time-interval, from pro-oestrus to early oestrus, it is not likely that this inactivation of the DA neurons resulting in decreased DA release by itself triggers off ovulation. Instead it seems likely that this decrease in DA release removes an inhibitory influence from the LHRF and FSHRF storing nerve terminals in the median eminence resulting in a facilitation of discharge of LHRF and FSHRF induced in turn via the activation of the preoptic-tubero tracts. As shown in the present study in rats with a persistent oestrous syndrome and no ovulation, there is no such removal of this inhibitory influence exerted by the DA neurons. A constant relatively high activity of the DA neurons could therefore contribute to the mechanism responsible for the blockade of peak LHRF and FSHRF release.

In low doses, little or no effects were obtained with oestrogen in androgen-sterilized castrated rats in contrast to that found in normal castrated rats. This decrease in sensitivity to oestrogen in androgen-sterilized castrated rats with regard to responses in the tubero-infundibular DA neurons may be due to a decrease in the uptake and accumulation of oestrogen into the hypothalamus. This has in fact been demonstrated by McEwen & Pfaff (1970). However, it should be pointed out that there is no marked reduction in responses of the tubero-infundibular DA neurons to oestrogen after androgen-sterilization. In agreement with our hypothesis that the DA neurons mediate the negative feed-back of oestrogen and testosterone on gonadotrophic secretion, it has also been demonstrated that oestrogen and testosterone in androgen-sterilized castrated rats can induce an inhibitory feed-back on the post-castration rises in FSH and LH secretion (van Rees & Gans 1966). Local application of testosterone and oestrogen to the brain indicate that the nuc. arcuatus is the site of negative feed-back (Flerkó & Szentagothai 1957; Davidsson & Sawyer 1961; Lisk 1960, 1962) i.e. where the tubero-infundibular DA neurons are also localized. It has also been demonstrated that there are oestradiol concentrating neurons in the arcuate nucleus (Pfaff 1968; Stumpf 1970). Thus, the present results with sex hormones are in agreement with our hypothesis that the tubero-infundibular DA neurons can partly mediate the inhibitory feed-back.
of oestrogen and testosterone on FSH and LH secretion. Progesterone, on the other hand, has practically no effects on the tubero-infundibular DA neurons (Fuxe et al. 1969a). Since progesterone seems at least partly to suppress LH release by interfering with the LHRF induced LH release in the pituitary gland (Spies et al. 1969; Arimura & Schally 1970) this lack of effect of progesterone on the DA neurons is also in agreement with our hypothesis.

The present results are in marked contrast to the view of McCann, Porter and coworkers that the DA in the median eminence acts by triggering off the release of LHRF and FSHRF since intraventricular injections of DA increases plasma LH (Schneider & McCann 1969, 1970) and LHRF and FSHRF in the portal blood (Kamberi et al. 1969, 1970a). However, these elegant studies are subject to criticism since the administration of DA into the median eminence area may as well affect also NA and 5-HT nerve terminals and other structures in this area. The effects of DA administered in this way may very well be different from physiologically released DA. Furthermore, the specificity of these effects has not been checked by studying a possible interference also with the regulation of TSH, STH and ACTH secretion. The view (Schneider & McCann 1970) that oestradiol blocks the dopaminergic release of LHRF, based on intraventricular injection of oestradiol previous to the DA injection, is also in marked opposition to the present and previous turnover studies (Fuxe et al. 1969a), which demonstrate the opposite, i.e. an increased release of DA after treatment with oestradiol. It is obvious, however, that at the present time no final explanation can be given for the difference in results obtained by our approach and by that of other groups of investigators.

Recently, data have been obtained that the tubero-infundibular DA neurons also participate in the regulation of prolactin secretion from the anterior pituitary. Thus, an increase in endogenous and exogenous prolactin secretion will cause selective increases in DA turnover in the median eminence, but not in the neostriatum, particularly of hypophysectomized rats (Fuxe & Hökfelt 1970; Hökfelt & Fuxe, in press; Olson et al., in press). These findings suggest that the DA neurons can also partly mediate the negative feed-back of prolactin on its own secretion, probably by increasing prolactin inhibitory factor (PIF) secretion (Clemens & Meites 1967, 1968). This view is supported by the fact that potent DA receptor blocking agents such as fluphenazine and perphenazine increase prolactin secretion (see Sulman 1970) and intraventricular injections of DA have been found to cause an increase of prolactin inhibitory factor secretion into the portal vessels (Kamberi et al. 1970b). Thus, at the same time as the tubero-infundibular DA neurons inhibit FSHRF and LHRF secretion they might also simultaneously enhance the secretion of PIF from the median eminence. The switch-off of the DA system in pro-oestrus could therefore be partly responsible for the simultaneous peak of FSH, LH and prolactin during pro-oestrus (Gay et al. 1970). Work is in progress in order to show whether
this hypothesis is valid. At the present time, however, it cannot be excluded that (1) the DA system is primarily involved only in the regulation of FSH-LH secretion, (2) the DA system is only involved in the regulation of prolactin secretion. If the former is true, the prolactin induced activation of the DA system would only represent one mechanism by which prolactin could reduce FSH-LH secretion (see also Fuxe & Hökfelt 1970), thus explaining the reverse relationship found between prolactin and FSH-LH secretion in many endocrine conditions.

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