THE EFFECT OF PITUITARY TRANSPLANTS ON THE TUBERO-INFUNDIBULAR DOPAMINE NEURONS IN VARIOUS ENDOCRINE STATES

By

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ABSTRACT

The effect of pituitary transplants into the anterior chamber of the eye of the rat on the amine turnover in the tubero-infundibular dopamine (DA) neurons has been studied by means of the Falck-Hillarp fluorescence technique and a tyrosine hydroxylase inhibition test. Transplantation was performed after hypophysectomy and/or castration, and in normal rats of both sexes. It was found that pituitary transplants caused an increase in DA turnover in the median eminence, particularly in hypophysectomized rats. The effects were cleargut as early as after 2 hours and the acceleration of DA turnover was partly reduced by treatment with 2 Br-a-ergokryptin or ergocornine. In normal cycling rats the cyclic DA turnover changes disappeared and a constant fairly high DA turnover, similar to that in dioestrus, was found. It is suggested that the DA turnover changes found are due to an increased endogenous secretion of prolactin and that in normal animals the DA neurons in the median eminence can partly mediate the inhibitory feedback action of prolactin on its own secretion by increasing the release of prolactin inhibiting factor (PIF) from the median eminence via an axo-axonic effect.

Testosterone and oestrogen, but not progesterone, cause a marked increase in the dopamine (DA) turnover in the median eminence but not in the neostriatum (Fuxe et al. 1967, 1969a). Furthermore, prolactin is capable of inducing a marked increase in DA turnover in the median eminence, particularly in hypo-
physsectomized rats (Fuxe & Hökfelt 1970; Hökfelt & Fuxe 1972). FSH and LH have been found to have no effect on the tubero-infundibular DA neurons.

The results with prolactin suggested that the tubero-infundibular DA neurons might participate in the control of prolactin secretion from the anterior pituitary. We have therefore continued these studies by the use of pituitary transplants into the anterior chamber of the eye. Pituitary transplantation is a widely accepted means for inducing endogenous prolactin secretion. The rat secretes high amounts of prolactin but very little of the other anterior pituitary hormones (see Everett 1954, 1956; Meites et al. 1963; Dao & Gawlak 1963). It was of primary interest to see if endogenously secreted prolactin was capable of causing an increased turnover in the DA neurons of the median eminence in intact, castrated, hypophysectomized and hypophysectomized + castrated rats.

MATERIAL AND METHODS

Animals

About 150 mature male and mature virgin female albino Sprague-Dawley rats (Anticimex, Stockholm), weighing 150-250 g, were used. They were housed at a temperature of 24 ± 1°C in a room in which the light went on by automatic control from 6 a.m. to 8 p.m.; the animals were maintained on a semi-synthetic diet and given water ad libitum. Male rats were used as pituitary donors.

Transplantations

Homologous transplantation of the anterior pituitaries took place when the animals were about 2 months old. The donor rats were killed by decapitation under ether anaesthesia and two 1/8-pieces of the anterior pituitary were transplanted into the anterior chamber of each eye. Thus, each animal received a total of half a pituitary. In some control animals a comparable amount of the submaxillary gland was transplanted into the anterior chamber of the eye. Transplantations were performed under sterile conditions using specially prepared instruments and a dissection microscope as described by Olson & Malmfors (1970).

The conditions of the transplants were followed in vivo by direct observation through the corneae of the slightly anaesthesized animals. All the transplants survived and rapidly became vascularized from the irides of the host eyes. No adverse immunological reactions were observed. The cycling female rats used for the transplantations were checked for 14 days by daily smears before the day of transplantation. Only female rats with regular 4 day cycles were used. All the transplantations took place during metoestrus and dioestrus. It has been shown that the stage of the ovarian cycle at transplantation does not influence the effect of the transplant (Nikitovitch-Winer & Everett 1958).

Experimental groups

The eight different groups that received pituitary transplants represented four different endocrine states each of which was studied in female and male rats. For the
numbers of animals and times between the operations and transplantations in the various groups, see Table 1. The control groups without pituitary transplants were run simultaneously (see Table 1). Hypophysectomy was performed via the parapharyngeal approach, orchidectomy by a ventral incision of the scrotum and ovariectomy by a dorsal abdominal incision. The hypophysectomized animals were given glucose 5% saline 0.9% solution as drinking water. Most of the rats were sacrificed 14 days after transplantation. In a series of rats the time course of the changes was followed.

In order to establish further whether prolactin released from the transplanted pituitaries was responsible for the effects observed in the median eminence, 2-brom-α-ergokryptin (CB154) and ergocornine were given to hypophysectomized rats 72 hours before sacrifice in a dose of 3 mg/kg ip. This drug is known to terminate pseudo-pregnancy, early pregnancy and lactation (Carlsen et al. 1961; Zeilmaker & Carlsen 1962) probably by decreasing prolactin secretion from the anterior pituitary (Flückiger & Wagner 1968; Yanai & Nakasawa 1970).

Fluorescence histochemical analysis of DA turnover

DA turnover in the median eminence was studied with the help of amine synthesis inhibition. The tyrosine hydroxylase inhibitor α-methyl-meta-tyrosine-methyl ester hydrochloride (H44/68) was given in a dose of 250 mg/kg intraperitoneally 2 1/2 hours before sacrifice. It is known that the rate of decline of the catecholamine (CA) stores following amine synthesis inhibition is highly dependent on the nervous impulse flow (Andén et al. 1969). It has recently been demonstrated that various endocrine states do not influence the degree of synthesis inhibition (Jonsson et al., to be publ.). Thus the degree of synthesis inhibition with H44/68 seems to be the same in normal, castrated and hypophysectomized rats. In the present experiments, therefore changes observed in the rate of disappearance of DA in the median eminence following H44/68 probably reflects changes in the amine turnover in these neurons. Furthermore, it is unlikely that the turnover changes observed are secondary to H44/68 induced changes in gonadotrophin secretion in view of the short time of action of H44/68, a period during which the pharmacological effects of H44/68 have not appeared. Possibly slight changes induced by H44/68 would also be present in the control groups. The purpose of the present investigation is only to evaluate any possible differences in turnover between experimental and control groups, and not to obtain absolute measurements of amine turnover.

The rats were killed by decapitation under chloroform anaesthesia. The hypothalamus was removed for histochemical fluorescence analysis of CA (Falck et al. 1962; Hillarp et al. 1966; Corrodi & Jonsson 1967; Olson & Ungerstedt 1970).

A semi-quantitative estimation of the fluorescence intensity in the DA nerve terminals in the external layer of the median eminence was performed on coded slides, often by two investigators independent of one another. It is known that a change in fluorescence intensity represents a change in amine levels (Olson et al. 1968; Jonsson 1969). Each fluorescence value represents the mean of 4–7 estimations of the fluorescence intensity in various parts of the median eminence from one animal. It was found that the various parts of the median eminence reacted similarly in the same animal. In contrast to what happens with peripheral adrenergic neurons, the fluorescence concentration relationship seems to be linear, probably up to normal levels in the DA neurons (Lidbrink & Jonsson 1971; and unpubl. data from this laboratory). A biochemical determination of the DA stores in the median eminence could not be performed as the methods available are not sufficiently sensitive.
Table 1.
The effect of pituitary transplants on the H44/68 induced DA disappearance in the tubero-infundibular DA neurons in various endocrine states.

<table>
<thead>
<tr>
<th>Endocrine state¹</th>
<th>Pituitary transplant²</th>
<th>Fluorescence intensity after H44/68 treatment³</th>
<th>Effects on H44/68 induced fluorescence disappearance compared with H44/68 treated non-transplanted controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact male</td>
<td>no</td>
<td>2 ± (8)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>female, dioestrus</td>
<td>yes</td>
<td>1¹/₂ ± (3) 2 + (4)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>female, pro-oestrus</td>
<td>no</td>
<td>1 + (7)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>2 + (2)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>1/2 ± (5) 1 + (3)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>Castration⁴</td>
<td>male (6 months)</td>
<td>no</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>1/2 ± (1) 1 + (5) 1¹/₂ + (1)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>male (3 weeks)</td>
<td>yes</td>
<td>1 + (3) 1¹/₂ + (2)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>female (6 months)</td>
<td>no</td>
<td>2 + (5)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>1 + (4)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>female (3 weeks)</td>
<td>yes</td>
<td>1 + (3) 1¹/₂ + (1)</td>
<td>no certain acceleration</td>
</tr>
<tr>
<td>Hypox male</td>
<td>no</td>
<td>3 ± (4)</td>
<td>marked acceleration reduction of transplant-induced acceleration</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>0 (5)</td>
<td>marked acceleration reduction of transplant-induced acceleration</td>
</tr>
<tr>
<td></td>
<td>yes + CB 154⁵</td>
<td>1 + (2) 2 + (2)</td>
<td>marked acceleration reduction of transplant-induced acceleration</td>
</tr>
<tr>
<td></td>
<td>yes + ergocornine⁵</td>
<td>1 + (3)</td>
<td>marked acceleration reduction of transplant-induced acceleration</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>male</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
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<td>------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>yes</td>
<td>yes + CB 154</td>
</tr>
<tr>
<td></td>
<td>3 + (5)^6</td>
<td>0 (5) 1/2 + (1)</td>
<td>1 + (3)</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Hypox + castration</td>
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</tr>
<tr>
<td>male</td>
<td>no</td>
<td>yes</td>
<td>yes (6 d)</td>
</tr>
<tr>
<td></td>
<td>3 + (11)^6</td>
<td>0 (4)</td>
<td>0 (4) 1/2 + (2)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>marked acceleration</td>
</tr>
<tr>
<td>female</td>
<td>no</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 + (6)^6</td>
<td>0 (4)</td>
<td></td>
</tr>
</tbody>
</table>

1) Rats were hypophysectomized (hypox) 2–4 weeks before transplantation. In these rats castration was performed on the day of transplantation.

2) Transplantation was performed by putting 2/3 of the pituitary gland into each anterior chamber of the eye. All rats were treated with H44/68, 250 mg/kg, ip, 2 1/2 hours before sacrifice. Most rats were sacrificed 14 days after transplantation. Exceptions to this are given within brackets.

3) A semi-quantitative estimation of fluorescence intensity was performed: 0 = no fluorescence, $\frac{1}{2}$ = very weak fluorescence, 1 + = weak fluorescence, 2 + = moderate fluorescence, 3 + = fairly strong fluorescence, 4 + = strong fluorescence. The evaluations were made on coded slides. If it could not be decided whether an observed fluorescence intensity belonged to one intensity grade or the next, this was indicated by adding half a plus to the lower grade. Numbers in brackets indicate number of animals studied.

4) The castration was performed 6 months or 3 weeks before sacrifice as indicated in the brackets.

5) CB 154 and ergocornine (3 mg/kg, ip) were given 72 h before sacrifice.

6) Hypophysectomy, with or without castration, causes a deceleration of the H44/68 induced fluorescence disappearance as compared to normal rats (Fuxe & Hökfelt 1970).
Histology

Samples of pituitary transplants, ovaries and vaginae from various groups were taken for routine histological examination. The gross appearance of the uterus was evaluated in all the rats.

RESULTS

The results are given in detail in Table 1. In a few castrated and hypophysectomized rats (3 of each) the effect of pituitary transplants was studied on the DA neurons without H44/68 treatment. No changes in the fluorescence intensity of the DA nerve terminals in the median eminence were found. The majority of rats were treated with H44/68. The main findings of these studies can be summarized as follows.

Intact male rats. – No definite effects on the disappearance of fluorescence from the DA nerve terminals in the median eminence were observed.

Intact female rats. – No cyclic activity in the median eminence such as found in the control group was observed. All the rats studied with anterior pituitary transplants showed an increased disappearance of DA fluorescence in the median eminence as compared to normal cycling controls in dioestrus, a stage of the cycle at which the activity of the system is at a maximum (Ahrén et al. 1971; Fuxe et al. 1972). The transplant did not influence the rate of DA fluorescence disappearance in the neostriatum. The vaginal smears showed a constant dioestrous appearance and the ovaries showed signs of increased luteinization in the histological sections.

Castrated female or male rats. – An increased disappearance of DA fluorescence was observed in the median eminence. Similar results were obtained in the rats castrated 6–7 months before transplantation and in those castrated 3 weeks before transplantation. No definite changes in the disappearance of fluorescence from the hypothalamic noradrenaline (NA) nerve terminals or from the neostriatal DA nerve terminals could be observed as a result of transplantation.

Fig. 1.
Hypophysectomized female rat. H44/68 treatment as described in text. The rats were operated 1 month before sacrifice. A fairly strong fluorescence is observed in the external layer of the median eminence (3 + in Table 1). V = third ventricle. × 250.

Fig. 2.
Hypophysectomized female rat with a pituitary transplant in the anterior chamber of the eye. Hypophysectomy was performed 1 month before sacrifice, and the transplantation took place 14 days before sacrifice. H44/68 treatment as described in the text. Practically no fluorescence is observed in the external layer of the median eminence (O in Table 1). V = third ventricle. × 250.
Hypophysectomized female or male rats with or without castration. – In both male and female hypophysectomized rats transplantation of pituitary glands induced a marked acceleration of DA disappearance from the external layer of the median eminence (Figs. 1 and 2). No definite changes in the disappearance of fluorescence from the hypothalamic NA nerve terminals or from the neostriatal DA nerve terminals were observed. Castration did not influence this effect of the pituitary transplants on the DA nerve terminals of the median eminence. The time curve showed that the effects were already marked after 24 hours, and that after 48 hours practically no fluorescence remained in the median eminence following treatment with H44/68. Pretreatment of hypophysectomized rats with CB 154 or ergocornine (72 hours before killing) caused a reduction of the increase in fluorescence disappearance induced in these animals by the pituitary transplants.

Histology
Sections of ovaries from hypophysectomized rats with long term pituitary transplants demonstrated a luteolytic action induced by prolactin as observed in previous studies (cf. Sloan & Malven 1969; Malven 1969). Histological sections from the transplants themselves revealed in practically all the groups an increased number of acidophilic cells.

The uteri of normal female rats having transplants all exhibited a dioestrous appearance. Hence, the uterine horns were thin and pale and the epithelium was low.

DISCUSSION
In previous studies it was found that exogenous prolactin can cause a selective increase in DA turnover in the median eminence (Fuxe & Hökfelt 1970; Hökfelt & Fuxe 1971), particularly in hypophysectomized rats. LH, FSH, ACTH and vasopressin did not cause any observable effect on the DA turnover in the median eminence. These results suggested that the tubero-infundibular DA neurons may inter alia be involved in the control of prolactin secretion from the anterior pituitary. Results from the present work further emphasize this view. Thus, it was found that pituitary transplants were capable of inducing a marked increase in DA turnover in the median eminence, particularly in the hypophysectomized rats. It is known that pituitary transplantation results in an increased prolactin release (Everett 1954, 1956; Meites et al. 1963; Dao & Gawlak 1963; Chondary & Greenwald 1967; Chen et al. 1970). The secretion of all the other hypophyseal hormones is considerably reduced. A conclusion that may be drawn from the present findings and the studies with exogenous prolactin is that the feed-back action of prolactin on its own secretion via
the increase of the prolactin inhibiting factor (PIF) secretion (Clemens & Meites 1967) is partly mediated via an increased release of DA in the median eminence.

The amine synthesis inhibition method is a well accepted method for the study of monoamine turnover (Andén et al. 1969). However, it is necessary to exclude that the effects observed are due to a change in the metabolism of the inhibitory drug injected. This is easily done in our experiments, since the effects observed were selective. Thus, there was an increase in DA turnover only in the median eminence. No turnover changes were observed in the DA nerve terminals of the neostriatum or in the hypothalamic NA nerve terminals after pituitary transplantation. So far, it has not been possible to detect any change in the degree of synthesis inhibition after H44/68 treatment in castrated or hypophysectomized rats, indicating that the inhibitory activity of the drug is not influenced by marked changes in the various endocrine states. Furthermore, since it seems almost impossible to induce more than about 80% of inhibition of the enzyme activity (Jonsson et al., to be published), an increased disappearance is probably under all circumstances not induced by an increased degree of inhibition of the enzymes.

As indicated above, it seems likely from the present experiments that an endogenous release of prolactin from the pituitary transplants was responsible for the increased turnover observed in the DA nerve terminals in the median eminence. Signs of increased prolactin release were also seen in the histological sections of the ovaries. Thus, ovaries from intact females showed signs of increased luteinization and those from the hypophysectomized rats showed signs of structural luteolysis as previously also observed by Piacsek & Meites (1967). This effect has been shown to be blocked by continuous ergocornine treatment (Malven & Hoge 1971), the mechanism probably being inhibition of prolactin release.

It is not likely that the effect of prolactin on the DA turnover in the median eminence was due to a direct amphetamine-like action on the DA nerve terminals, since amphetamine, a potent releaser of extragranular catecholamine stores (Carlsson et al. 1966; Fuxe & Ungerstedt 1970), does not increase the disappearance of DA after synthesis inhibition (Corrodi et al. 1967) as found with prolactin, nor are blockers of CA uptake capable of increasing the disappearance of amine stores after synthesis inhibition (Corrodi et al. 1967). It therefore seems probable that the marked increase in DA turnover observed in the hypophysectomized rats is due to an increased nervous activity in the tubero-infundibular DA neurons. It is not possible to say if this action is induced by a direct effect of prolactin of the DA cell bodies or if it is an indirect effect.

One of the actions of 2-brom-α-ergokryptin and ergocornine is to decrease prolactin secretion from the anterior pituitary (Yanai & Nakasawa 1970;
Malven & Hoge 1971; Wuttke et al. 1971). The fact that this drug decreases the increase in DA turnover induced by the pituitary transplants therefore supports the view mentioned above, that prolactin is responsible for the observed effects on DA turnover in the median eminence. The time-course experiment also supports this view, since marked activation of the DA neurons was observed 24 hours after the transplantation of the pituitaries. It is known that the secretion of prolactin after removal of the inhibitory influence of the hypothalamus is markedly increased after 24 hours (see monograph by Sulman 1970).

Recent evidence from the Meites group clearly suggests that prolactin regulates its own secretion via a short feed-back mechanism. Thus, in studies on the effect of prolactin implants into the median eminence on PIF secretion, they found that prolactin increased the hypothalamic contents of PIF, reduced pituitary prolactin concentrations and induced atrophy of the mammary glands. Furthermore, an implant of prolactin into the median eminence was found to reduce lactation and to shorten the length of pseudo-pregnancy (Clemens & Meites 1968, 1969; Chen et al. 1968; Mishkinsky et al. 1969; see also review of Meites 1970). Pituitary transplants in intact and ovariectomized female rats can also significantly influence the host pituitary prolactin (Welsch et al. 1968).

In view of the present findings and the fact that exogenous prolactin also markedly increases DA turnover in the median eminence (Hökfelt & Fuxe 1971), it is suggested that DA partly mediates the short feed-back of prolactin on PIF secretion. It is possible that the DA released by prolactin stimulation acts by increasing the secretion of PIF into the hypophysyal portal vessels. This view is also supported by the fact that neuroleptic drugs, which interfere mainly with DA transmission, cause a marked increase in prolactin secretion (Fuxe 1970; see also Sulman 1970). It seems clear that the capacity of neuroleptic drugs to induce prolactin secretion is related to their ability to block central DA receptors (Sulman 1970; Fuxe 1970; Andén et al. 1970). Furthermore, Porter's group has recently found that the injection of DA into the third ventricle results in an increased secretion of PIF (Kamberi et al. 1970). All these data taken together clearly support the view that DA is partly mediating the feed-back of prolactin on its own secretion. An increased released of DA results in an increased secretion of PIF. It is possible therefore that the high DA turnover found in pregnancy and pseudopregnancy (Fuxe et al. 1969b) could be partially responsible for the low serum prolactin levels found in these states (Amenomori et al. 1970).

It is interesting that the activation of the DA neurons by the transplants was much more marked in hypophysectomized rats than in castrated or normal intact rats. This is difficult to explain, but it is probably related to the fact that in castrated animals there are other factors regulating the activity of the
DA neurons and the high levels of LH and FSH present in this state could interfere with the action of prolactin. In intact animals the situation is even more complicated by the presence of gonadal steroid hormones. The fact that an action was observed in normal female rats but not in normal male rats can be related to the fact that oestrogen probably increases directly the secretion of prolactin from the transplants (cf. Nicoll & Meites 1962; Zeilmaker 1970).

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REFERENCES


Clemens J. A. & Meites J.: Physiologist 10 (1967) 144.
Clemens J. A. & Meites J. Endocrinology 82 (1968) 878.

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