RECENT MORPHOLOGICAL AND FUNCTIONAL STUDIES ON HYPOTHALAMIC DOPAMINERGIC AND NORADRENERGIC MECHANISMS

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DURING recent years there has been an ever increasing interest in the role that hypothalamic catecholamines (CA) play in regulating anterior pituitary function. We have been particularly concerned with the functional role of the tubero-infundibular dopamine (TIDC) neurons (FUXE *et al.*, 1967; FUXE and HÖKFELT, 1969; FUXE *et al.*, 1970; HÖKFELT and FUXE, 1972). The evidence so far obtained suggest that the TIDA neurons mainly are involved in the control of gonadotrophin secretion (FUXE *et al.*, 1967; FUXE and HÖKFELT, 1969; HÖKFELT and FUXE, 1972; SCHNEIDER and MCCANN, 1970; KAMBERI *et al.*, 1970). The groups have agreed that the dopamine (DA) in the median eminence may enhance the secretion of prolactin inhibitory factor (PIF) (see also MEITES *et al.*, 1972). Our own work based on turnover studies of the TIDA neurons in various endocrine states and on pharmacological work suggest that the TIDA neurons also could inhibit luteinising hormone releasing factor (LRF) and possibly follicle stimulating hormone releasing factor (FRF) secretion (FUXE *et al.*, 1969a, b; FUXE *et al.*, 1970; HÖKFELT and FUXE, 1972). Studies using intraventricular injections of CA, on the other hand, have suggested that DA could increase the secretion of LRF and FRF (SCHNEIDER and MCCANN 1970; KAMBERI *et al.*, 1970). The present paper will mainly review recent new work from our group that relate to the functional role of the CA neurons.

MORPHOLOGY

Immunohistochemical studies (HÖKFELT *et al.*, 1973)

Dopamine-β-hydroxylase. Using antibodies against dopamine-β-hydroxylase (DβH) it has been possible to discover DβH containing, and thus probably noradrenergic, terminals in certain parts of the lateral external layer of the median eminence. These terminals appeared as a thin dotted zone of specific immunofluorescence immediately adjacent to the primary capillary plexus. Scattered DβH containing terminals were also found in the medial external part which is rich in capillary loops. These latter DβH containing terminals seem to correspond to those CA terminals discovered with the Falck-Hillarp technique, to have a very slow disappearance of fluorescence following tyrosine-hydroxylase inhibition. In agreement with previous pharmacological work (see FUXE and HÖKFELT, 1969; JONSSON *et al.*, 1972) the DβH containing nerve terminals in the subependymal and fibre layer of the median eminence appeared to have a similar distribution and frequency as the CA nerve terminals in these areas.

The immunohistochemical evidence therefore underline the view that the majority of the CA nerve terminals in the subependymal zone and the fibre layer, where they appear as large droplets, contain noradrenaline (NA), whereas the majority of the

CA nerve terminals of the external layer of the median eminence contain DA (see HÖKFELT and FUXE, 1972; JONSSON *et al.*, 1972; BJÖRKLUND *et al.*, 1973). On the other hand, it should be underlined that using DβH immunofluorescence technique, a new NA system has been discovered in the external lateral layer close to the primary capillary plexus. These terminals, although few compared to the DA terminals, could play an important functional role. It has to be considered that these NA terminals can mediate the LRF releasing activity of intraventricularly administered DA (SCHNEIDER and MCCANN, 1970) and adrenaline (RUBINSTEIN and SAWYER, 1971).

The lack of positive phenylethanolamine-*N*-methyl-transferase immunofluorescence in these terminals indicate that they do contain NA and not adrenaline. The findings imply that NA nerve terminals in a certain restricted part of the median eminence can exert an axo-axonic influence on peptidergic nerve terminals containing releasing or inhibitory factors.

Studies on dopa-decarboxylase. The results support the view given above from the experiments with DβH. With our present antibodies and technique, the amounts of dopa-decarboxylase (DDC) in the NA nerve terminals are too low to be demonstrated, and in agreement positive DDC immunofluorescence was only found in the external layer, especially in the lateral part and in cell bodies in the arcuate nucleus.

Hypothalamic islands (JONSSON *et al.*, 1972)

These studies demonstrated that the DA nerve terminals in the external layer mainly derived from DA cell bodies in the arcuate nucleus and the anterior periventricular nucleus, ventral part. Thus, the DA nerve terminals remained unchanged in complete hypothalamic islands, in which all other CA cell body groups except A12 were excluded from the island including groups A11 and A13 and CA cell bodies in the most anterior part of the periventricular hypothalamus. Recent results obtained by BJÖRKLUND *et al.* (1973) are in agreement with this view. The NA nerve terminals in the internal layer including the subependymal layer probably derive from ascending NA tracts from the pons and the medulla oblongata, since they are not present in hypothalamic islands (JONSSON *et al.*, 1972; see also BJÖRKLUND *et al.*, 1973).

Pharmacological analysis

In vitro studies using the neurotoxic compound 6-hydroxy-dopamine (6-OH-DA) (JONSSON *et al.*, 1972) revealed that a resistance of the CA nerve terminals in the external layer and a disappearance of the CA nerve terminals in the internal layer is correlated with an intact DA synthesis and blockade of the NA synthesis, respectively, which is in good agreement with previous pharmacological work (see HÖKFELT and FUXE, 1972; JONSSON *et al.*, 1972).

Recent microfluorimetric studies with FLA 63 in our laboratory (LÖFSTRÖM, unpublished data) demonstrate a certain reduction of the fluorescence in the lateral part of the external layer but not in the neostriatum suggesting in agreement with the immunohistochemical work, that a minor part of these terminals may contain NA. The depletion was, however, greater in the internal and subependymal zone in agreement with previous work (CORRODI *et al.*, 1970).

It should be underlined that although the dopaminergic mechanism in the median eminence probably represent the major one in neuroendocrine control, there are DA

nerve terminals in various limbic structures, which could represent important extrahypothalamic sites for dopaminergic control of hypothalamic function. It is known that e.g. fornix transection strongly depresses pituitary-gonadal activity (KAWAKAMI *et al.*, 1972). This idea is also favoured by the fact that our group has recently been able to confirm the presence of new types of DA nerve terminals (THIERRY *et al.*, 1973) e.g. in the limbic cortex especially in the gyrus cinguli. These studies are based on a special pharmacological model utilizing the occurrence of selective reserpine resistant binding of CA in the DA neurons (LIDBRINK *et al.*, 1973; HÖKFELT *et al.*, 1973) in combination with the Vibratome sectioning technique (HÖKFELT and LJUNGDAHL, 1972; LINDVALL *et al.*, 1973).

Although many findings support the idea of an action of CA mainly at the median eminence level, an action at the pituitary level in addition should not be overlooked. Thus, several workers (MACLEOD *et al.*, 1970; KOCH *et al.*, 1970) have found that CA produce a dose-dependent reduction of prolactin release *in vitro* from anterior pituitaries. These results have to be taken into account as well when trying to explain neuroendocrine effects of monoaminergic drugs. E.g. it is also known that dopa can be taken up and decarboxylated by PAS positive cells in the anterior pituitary (DAHLSTRÖM and FUXE, 1966; HÅKANSON *et al.*, 1972).

FUNCTION

During a number of years we have studied the turnover changes that occur in the DA nerve terminals in the external layer of the median eminence in various endocrine states and after treatment with hormones and psychoactive drugs. Changes in turnover have been evaluated by studying changes in disappearance of fluorescence after treatment with the tyrosine-hydroxylase inhibitor, α-methyl-tyrosine methylester. The pattern of fluorescence disappearance is shown in Fig. 1. It can be seen that there is good agreement between semiquantitative subjective estimations and the microfluorimetric measurements (LÖFSTRÖM, JONSSON and FUXE, unpublished data). The initial rapid decline should be noted, the degree of which may give an estimation of the turnover in a small functionally very active pool of amines. The partial restoration of fluorescence observed 30 min after H 44/68 (see GLOWINSKI, 1972) may possibly be explained by the fact that there is a continuous transport of amine granules from the cell bodies to the terminals and that the amine containing granules in the fibres are not depleted to any extent by H 44/68 treatment before they have reached the nerve terminals and can be released by the nervous impulse flow. From Fig. 1 it can be calculated that $T_{1/2}$ of the main amine pool is in the order of 135 min. In agreement with previous findings (FUXE *et al.*, 1969a) it can be seen that the turnover of the main amine pool is markedly increased in lactation and the calculated $T_{1/2}$ is in the order of 85 min. Also the DA levels appear to be somewhat reduced in lactation compared with normal male rats. In castrated female rats, on the other hand, $T_{1/2}$ of the main amine pool appears to be similar to that in normal male rats.

These fluorescence disappearance curves also clearly indicate that there seems to be a linear fluorescence–concentration relationship in the DA terminals, in contrast to the situation in other CA neuron systems (see JONSSON, 1971). In the future, it will be of interest to study particularly this initial decline of fluorescence, since the

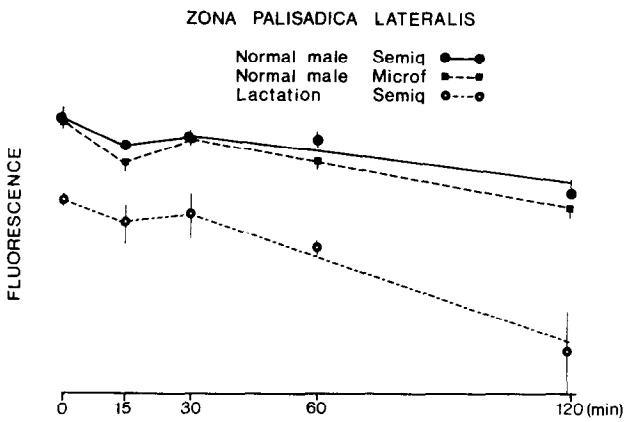

FIG. 1.—The time-curve of fluorescence disappearing after H 44/68 is illustrated in normal male rats and lactating female rats. In normal male rats both micro-fluorori-metric and semiquantitative estimations of fluorescence intensity have been made. Means ± S.E.M. are given.

the small functional active pool may be particularly sensitive to changes in nervous impulse activity (GLOWINSKI, 1972).

In our work (see FUXE and HÖKFELT, 1969; HÖKFELT and FUXE, 1972) we have so far mainly studied the turnover in the main pool in the lateral part of the external layer of the median eminence, and the 2-hr interval after H 44/68 has been found suitable for this purpose (see Fig. 1). The results can be summarised in the following way. In states with low FSH–LH secretion and blockade of ovulation there is an increase in DA turnover in the TIDA neurons, whereas during periods of high LH–FSH secretion and prolactin secretion such as in the "critical period" of adult female rats and of immature rats treated with PMS and in castrated animals, the turnover in the TIDC neurons is low (FUXE *et al.*, 1969a, b; AHRÉN *et al.*, 1971; FUXE *et al.*, 1972). It has also been discovered that estrogen, testosterone, clomiphene, stilbestrol and antifertility steroids of the estrogen type or the nortestosterone type under conditions in which they exert negative inhibitory feedback on gonadotrophin secretion, markedly increase turnover in the TIDA neurons (FUXE *et al.*, 1971a). A similar marked increase in turnover has also been observed after injection of prolactin into hypophysectomised rats. In view of these findings we have postulated that the TIDA neurons may act by inhibiting LRF–FRF secretion and enhancing PIF secretion.

Most of our previous work has mainly involved studies on the lateral part of the external layer. Therefore we have made a study to compare the effects of prolactin on the lateral and medial part of the external layer in hypophysectomised rats. The results are summarised in Fig. 2 (JONSSON, FUXE and LÖFSTRÖM, unpublished data). In the upper part of the figure it can be seen that there is a slight reduction of the fluorescence intensity after hypophysectomy (4 weeks) both in the medial and lateral part of the external layer. In the lower part of the figure the percentage disappearance of fluorescence is given at the 2 hr interval following H 44/68. A dramatic acceleration of fluorescence disappearance is observed after the prolactin injection (5 mg/kg i.v., 24 hr before killing). This dramatic effect occurs in both the

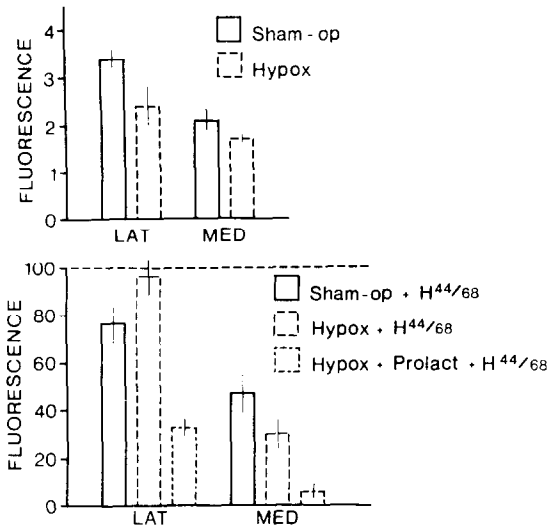

FIG. 2.—Effect of prolactin on the disappearance of fluorescence in hypophysectomised rats after H 44/68. Prolactin was given i.v. in a dose of 5 mg/kg 24 hr before killing. Hypophysectomy had been performed 4-weeks earlier. H 44/68 was given i.p. in a dose of 250 mg/kg 2 hr before killing. Microspectrofluorimetric measurements have been performed. The fluorescence in the upper part of the figure is given in fluorescence units and in the lower it is given as percentage of normal and hypophysectomised rats respectively. Means ± S.E.M.

medial and lateral part of the external layer. It should be noted, however, that there is a reduction of DA turnover in the lateral part after hypophysectomy, whereas the DA turnover may be increased in the medial part. These interesting observations, however, require further study but points out the importance of looking for possible regional changes in the various DA terminal systems in the median eminence.

Our hypothesis on the role of the TIDA neurons in gonadotrophin control has recently obtained support by studies on the turnover of the TIDA neurons under conditions of positive gonadal steroidal feedback and by pharmacological studies. Thus, it is know that in castrated estrogen primed female rats, progesterone will after certain time interval induce a peak of FSH, LH and prolactin (Caligaris *et al.*, 1971; KALRA *et al.*, 1972). It has now been possible to demonstrate that estrogen alone causes a significant increase of DA turnover in the palisade zone, which is not seen in combination with progesterone (LÖFSTRÖM, FUXE and HÖKFELT, unpublished data; see Table 1). Furthermore, the latter response pattern is not seen in androgen sterilised animals (see Table 2), which are known to have no cyclic gonadotrophin release (Barraclough, 1967). Recent studies with DA receptor stimulating agents, DA precursors and releasing agents such as ET 495, CB 154, dopa and amphetamine, demonstrate that marked increases in DA receptor activity will result in inhibition of ovulation in immature female rats (FUXE *et al.*, 1971b; HÖKFELT and FUXE, 1972; FUXE, HÖKFELT and LÖFSTRÖM, unpublished data). After treatment of normal castrated estrogen primed female rats with progesterone, turnover changes also occur in other monoamine neuron systems. Thus, using H 44/68 in combination with a histochemical and biochemical analysis of NA it has been found that progesterone increase NA turnover both in the hypothalamus and in the cortex cerebri (FUXE,

TABLE 1. TIDA NEURONS AND POSITIVE GONADAL STEROID FEEDBACK

Treatment	Fluorescence intensity after H 44/68 (Number of animals in parenthesis)				Statistical significance according to Tukey's quick test (Tukey, 1959 NEAVE and GRANGER, 1968)
Castration					
McCann model					
Oil		1·5(4)	2(2)	2·5(1)[a]	
Estrogen (5 μg)	1·5(3)	1(2)[b]			a–b: $P < 0{\cdot}01$
Estrogen (5 μg) + progesterone (1·5 mg)		1(1)	1·5(3)	2(1)[c]	a–c: N.S.

The fluorescence intensity scale varies from 0 to 4. 0 = no fluorescence; 1 = weak intensity; 2 = moderate intensity; 3 = strong intensity; 4 = very strong intensity. For semiquantiative estimation, see LIDBRINK and JONSSON (1971).

TABLE 2. TIDA NEURONS IN ANDROGENSTERILISED RATS: EFFECTS OF PROGESTERONE AFTER ESTROGEN PRIMING

Treatment	Fluorescence intensity after H 44/68 (Number of animals in parenthesis)			Statistical significance according to Tukey's quick test (TUKEY, 1959; NEAVE and GRANGER, 1968)
Androgensterilisation (1·25 mg testosteron-proprionate day 3) + castration				
McCann model				
Oil	1(2)	1·5(6)	2(2)[a]	
Estrogen (5 μg)	1(3)	1·5(2)[b]		a–b: N.S
Estrogen (5 μg) + progesterone (1·5 mg)	1(5)	1·5(2)[c]		a–c: N.S.

For further details, see text to Table 1.

HÖKFELT, JONSSON and LÖFSTROM, unpublished data). These results support the view view that NA neurons in the hypothalamic and preoptic area as well as in the median eminence could facilitate the release of LRF and possibly FRF (KALRA *et al.*, 1972). Interestingly enough the 5-HT turnover changes as revealed by biochemical analysis of 5-HT and by the use of α-propyldopacetamide, a tryptophane hydroxylase inhibitor, are in the opposite direction. Thus, the 5-HT turnover increases after estrogen priming alone, whereas the NA turnover was unchanged by this treatment (FUXE, HÖKFELT, JONSSON and LÖFSTRÖM, unpublished data). Furthermore, after progesterone treatment of the estrogen primed animals, the 5-HT turnover was reduced back to normal. These results are in good agreement with the view of KORDON *et al.* (1972) that there exist an inhibitory 5-HT pathway controlling LH secretion and also suggest that this 5-HT system could be involved in the inhibitory feedback action of estrogen on gonadotrophin secretion. In view of the above findings it can be speculated that the *TIDA neurons and the hypothalamic 5-HT neurons have to be* turned off, *whereas the hypothalamic NA neurons have to be* turned on *in the "critical period" in order for the LH, FSH and prolactin surge to occur resulting in ovulation.*

At the present time it cannot be excluded that the TIDA neurons are also involved in the control of other releasing and inhibitory factors beside those controlling gonadotrophin secretion, since certain turnover changes have been observed after stress (retardation; LIDBRINK *et al.*, 1972), thyroidectomy (acceleration; FUXE, LÖFSTRÖM and TSUCHIYA, unpublished data) and growth hormone in high doses (HÖKFELT, HALL and FUXE, unpublished data). At present, studies exploring a possible regional differentiation in these states are in progress. However, these effects are much less dramatic than the effects after interference with the pituitary-gonadal axis and could be interpreted to be related to changes in prolactin secretion. It should also be remembered that there probably exist dopaminergic mechanisms in other areas particularly in the limbic system that may control neuroendocrine events.

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