CATECHOLAMINES IN SEXUAL HORMONE REGULATION: FOREBRAIN INFLUENCE ON TUBERO-INFUNDIBULAR DOPAMINE NEURONS AND INTERACTION WITH CHOLINERGIC SYSTEMS

W. LICHTENSTEIGER
Department of Pharmacology, University of Zürich, Zürich, Switzerland

NEUROENDOCRINE systems are capable to produce a variety of fast responses, a fact that may be especially important with regard to the coordination of behavioural and hormonal processes. We have been interested in the question whether monoamine systems and in particular the tubero-infundibular dopamine (DA) neurons might come into play in such short-term adjustments.

STIMULATION-INDUCED CHANGES IN CELLULAR FLUORESCENCE INTENSITY AND THEIR POSSIBLE BIOCHEMICAL BACKGROUND

As a tool to detect rapid responses in the tubero-infundibular DA neurons, we used a characteristic short-term change in the intensity of the catecholamine fluorescence of their cell bodies. The latter was measured by a microfluorimetric technique based on the histochemical fluorescence method of Falck and Hillarp (LICHTENSTEIGER, 1969a, 1970, 1971). Various populations of central DA neurons of mice and rats were found to exhibit this acute change in intensity upon a number of treatments such as local electrical or transsynaptic stimulation, acute exposure to cold, morphine or physostigmine (Fig. 1; LICHTENSTEIGER, 1969b; 1971; HEINRICH *et al.*, 1971; LIENHART and LICHTENSTEIGER, 1973). The response is prevented by tyrosine hydroxylase inhibition which indicates that it is linked with an enhancement of DA synthesis. However, it appears that it is not due exclusively to the formation of the amine: Determinations of DA in extracts from substantia nigra-pieces of mice, carried out by means of a fluorimetric micromethod (SCHLUMPF, 1973), yielded an initial intensity change that was opposed to the one observed by microfluorimetry. Since the intensities of DA and DOPA are inversely related in the two procedures, we thought of a possible contribution of DOPA to the final fluorescence intensity. Although DOPA was not detected in extracts of normal whole brain (KEHR *et al.*, 1972), we have recently observed a band corresponding to the position of DOPA in thin layer chromatograms of extracts from mouse midbrain, where amines and DOPA were visualised by reaction with formaldehyde vapour. As a working hypothesis, we would suggest, therefore, that neuronal activation induces a transient shift in the proportion of DOPA vs. DA in the cell bodies which, together with the formation of DA, may account for the intensity changes.

INFLUENCE OF VARIOUS BRAIN REGIONS ON THE TUBERAL DA NEURONS AND INTERACTION WITH CHOLINERGIC SYSTEMS

With regard to the questions put forward in the introduction, it would seem to be important to have some information on the integration of the tuberal DA neurons

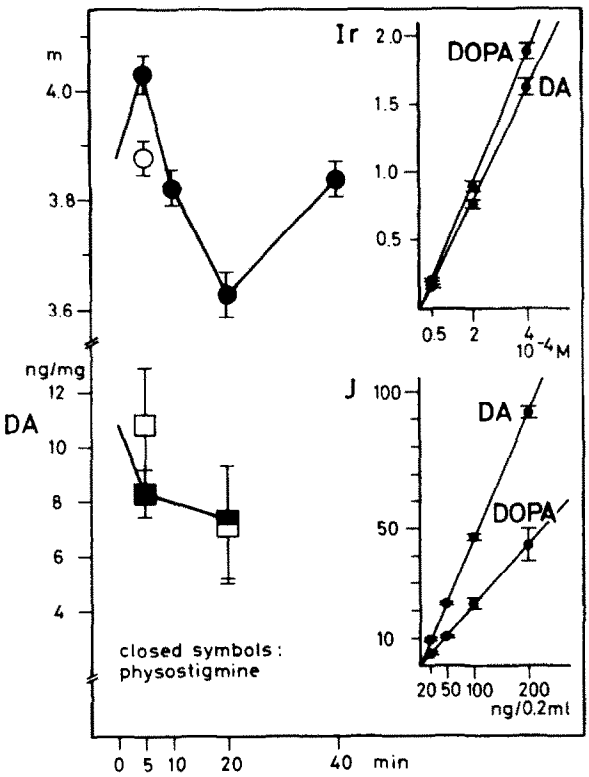

FIG. 1.—Acute response of substantia nigra DA neurons of mice to physoistgmine (0·25 mg/kg s.c.). *Upper half, left:* Biphasic intensity response in DA nerve cells as detected by microfluorimetry. Ordinate: means with 99% confidence limits (cell counts 763–803 per experimental group); abscissa time in min. *Upper half, right:* Relative intensities of DA and DOPA in 7 μ sections of gelatin standards, measured against a NA standard, after treatment according to the histochemical fluorescence method. Ordinate: means with 99% confidence limits (100 measurements per point); abscissa: DA and DOPA concentrations in the original 2% gelatin solution. *Lower half, left:* DA concentrations as determined by a fluorimetric micromethod in extracts of substantia nigra-pieces of mice subjected to the same experimental conditions. Ordinate: means with 99% confidence limits (9 assays per point each performed on the two blocks of substantia nigra of both sides from one animal); abscissa: time as above. *Lower half, right:* Intensities of DA and DOPA as found in the extraction method. Ordinate: means with 99% confidence limits (8 determinations per point) in absolute instrument values; abscissa: concentrations of DA and DOPA. The changes in fluorescence intensity observed with the two methods after the first 5 min as well as the relations of the fluorescence intensities of DA and DOPA are opposed to each other.

into the neuroendocrine organisation. In a search for regions capable of influencing the DA neurons, we used the initial increase in fluorescence intensity induced by stimulation as an evoked response. The experiments were mainly performed on ovariectomised rats pretreated for one day with estrogen and progesterone. A clearcut intensity response was elicited by intermittent electrical stimulation (10 min) of the medial preoptic area, nucleus of diagonal tract, ventrolateral part of the bed nucleus of stria terminalis, medial amygdaloid nucleus and ventromedial tegmental area (VMT) of the midbrain. Certain effects were also noted after stimulation of the ventral hippocampus (LICHTENSTEIGER, 1971, 1973 and in preparation). It

appears thus that the tubero-infundibular DA neurons (1) are capable of short-term responses and (2) may serve to transmit signals from higher-order neuroendocrine 'centers', *limbic structures* and *ascending brainstem systems*.

In most cases, the effect exerted on the tuberal DA neurons appeared to depend upon the activity of cholinergic systems (LICHTENSTEIGER, 1973): Atropine administered 15 min before the onset of electrical stimulation markedly reduced the response to stimulation in the medial preoptic area, nuc. of diagonal tract, bed nucleus of stria terminalis and midbrain VMT. The drug also exerted a moderate effect on the reaction to amygdaloid stimulation. The effect of atropine was most probably due to a specific action at some cholinergic synapse(s), since (1) local electrical stimulation in the arcuate nucleus, where most of the DA cell bodies are situated, was effective despite atropine treatment, (2) the reduction of the intensity response to electrical stimulation of the medial preoptic area was dose-dependent (range 0·4–10 mg/kg, s.c.) and (3) methylatropine administered s.c. in a dose that was equimolar to the highest dose of atropine used, was almost ineffective. The fact that the effect of the drug rather did not appear to be linked with a special site of stimulation, and also its complex interaction with hormone secretion, could mean that the cholinergic synapse(s) may not belong to a neuron of a specifically neuroendocrine pathway but rather, to a cholinergic projection exerting some facilitatory influence on the transmission of the stimulatory effect.

RELATIONSHIP TO LUTEINISING HORMONE (*LH*) AND PROLACTIN SECRETION: HOMOGENEITY OR HETEROGENEITY OF THE TUBERAL DA NEURON GROUP?

Whenever responses of tuberal DA neurons and hormonal changes are compared, one should take into consideration that this neuron group does not only project to the external layer of the median eminence but also to intermediary and probably neural lobes (cf. BJÖRKLUND *et al.*, 1973). For safe conclusions, it would be necessary to investigate simultaneously the various hormone axes. Our own information is limited to serum *LH* and prolactin which were determined by radioimmunoassay (LICHTENSTEIGER and KELLER, in preparation).

Despite these limitations, certain indications for functional differentiation within the DA neuron group became evident in our material: Thus, the magnitude of the intensity response to electrical stimulation varied through the antero-posterior extension of the arcuate nucleus (Fig. 2). Moreover, the extent to which the response was inhibited by atropine, also appeared to differ somewhat in the various parts of the nucleus. Topographical differences were further noted when intensity profiles of groups of stimulated rats with different *LH* concentrations ranges were compared. In view of such differences and in consideration of earlier findings (LICHTENSTEIGER, 1969b), the tuberal DA neuron group was divided into two parts (levels 1–7 and 8–15) and fluorescence intensities and hormone levels of individual rats were correlated separately for the two parts.

From these data, it appears that *two types of responses* may tentatively be considered. They are represented by the results obtained with stimulation of the medial preoptic area and of the medial amygdaloid nucleus (Table 1) : (1) *Medial preoptic stimulation* yielded a significant positive correlation between the increased fluorescence intensity of the *anterior* part of the DA cell group and an increase in *LH*

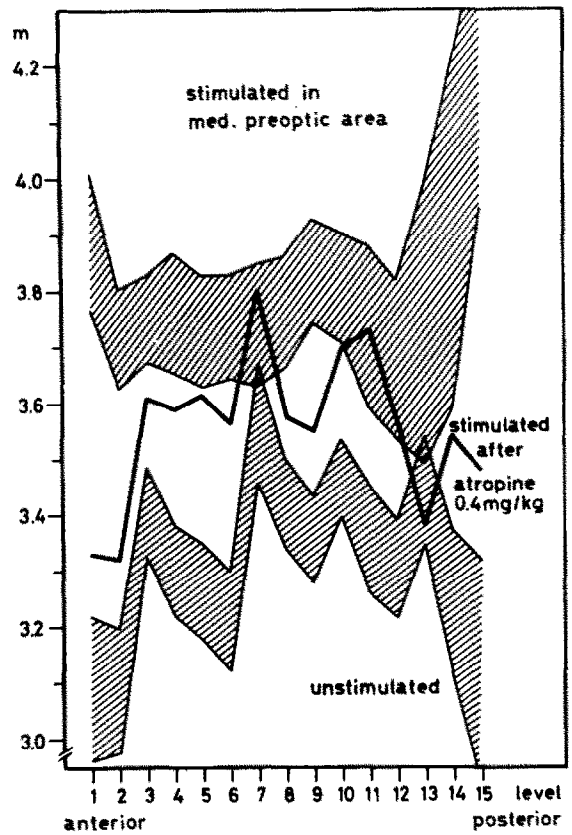

FIG. 2.—Profiles of fluorescence intensity of DA nerve cells through the arcuate nucleus of ovariectomised rats pretreated with estrogen and progesterone (5 rats per group). Abscissa: investigated levels, distance between levels $\sim 84\,\mu$. Ordinate: intensity of the catecholamine fluorescence in natural logarithms. The shaded areas indicate the 99% confidence limits of sham-operated controls (lower half) and animals stimulated for 10 min in the medial preoptic area (upper half). The magnitude of the increase in intensity differs at different levels, with most intense responses in the anterior and the posteriormost parts. The solid line connects the means of a group stimulated preoptically after atropine: It seems that the reduction of the stimulation-induced response is most marked at levels with greater intensity responses. All values are based on ~40–100 cells per level and group, except for levels 1,2,14,15 with lower cell counts.

concentration. No significant correlation was found with the posterior part, although the intensity increased there, too. This relation is basically in agreement with our earlier findings (KELLER and LICHTENSTEIGER, 1971). Atropine typically reduced both parameters, the correlation remaining thus positive. In contrast, *prolactin* remained largely unchanged after preoptic stimulation in the absence of atropine and accordingly, no significant correlation between intensity and hormone level was seen. Yet, atropine, while reducing the intensity response, allowed an increase in prolactin concentrations to occur upon preoptic stimulation. This led to a positive correlation between intensity and prolactin level (significant in both parts of the region). (2) After *medial amygdaloid stimulation*, the relation between *LH* and

TABLE 1. FLUORESCENCE INTENSITY OF THE ANTERIOR PART OF OF THE TUBERAL DA NEURON GROUP.
(Mean (m), variance (s^2), cell count (n) of logarithmically transformed frequency distributions of relative fluorescence intensity (natural logarithms) and serum concentrations of luteinising hormone (LH) and prolactin (determined by radioimmunoassay) of individual rats.

Electrode site and stimulation parameters	m	s^2	n	LH (ng/ml)	Prolactin (ng/ml)	Electrode site and stimulation parameters	m	s^2	n	*LH* (ng/ml)	Prolactin (ng/ml)
I. Medial preoptic area	3·230	0·1563	147	80	500	IV. Medial preoptic	3·399	0·1887	105	160	460
10 min, no stimulation	3·300	0·1651	75	290	100	area, 10 min, no stimu-	3·411	0·2568	162	250	420
	3·182	0·1381	81	170	420	lation, atropine	3·107	0·1898	115	200	420
	3·540	0·1549	82	195	—	10 mg/kg s.c. 15 min	3·207	0·1515	148	120	280
	3·193	0·2454	132	180	280	before electrode placement	2·974	0·1926	98	130	220
II. Medial preoptic area	3·853	0·1291	93	245	50	V. Medial preoptic	3·561	0·1262	98	250	—
10 min, 100 μA, 0·5 msec	3·602	0·1405	91	380	280	area, 10 min stimula-	3·278	0·2883	141	220	420
100 cs, 15 sec on/off	3·757	0·1329	76	360	160	tion with same param-	3·346	0·1819	94	250	350
	3·719	0·1362	59	230	280	eters as II., atropine	3·267	0·1551	91	220	460
	3·830	0·1854	73	310	460	10 mg/kg s.c. 15 min before onset of stimulation	3·409	0·1409	93	250	560
III. Medial amygdaloid	3·727	0·1458	92	140	560	VI. Medial amygdaloid	3·491	0·1703	92	280	—
nucleus, 10 min stimula-	3·521	0·1167	101	185	500	nucleus, 10 min stimula-	3·378	0·1753	99	250	560
tion, same parameters	3·481	0·1576	75	140	460	tion, same parameters	3·610	0·1405	106	—	560
as II.	3·659	0·1254	81	225	500	as II. atropine 10 mg/kg 15 min before onset of stimulation					

Correlation coefficients (a = r for anterior part, p = r for posterior part (intensities not shown); * = significant for $P < 0·05$): *Correlation of mean intensities with* LH *levels:* I and II a = 0·61*, p = 0·54; IV and V a = 0·67*, p = 0·38; II and V a = 0·47, p = 0·46; I and III a = 0·07, p = −0·07; IV and VI a = 0·71*, p = 0·58; III and VI a = −0·45, p = −0·47. *Correlation with prolactin levels:* I and II a = −0·33, p = −0·25; IV and V a = 0·71*, p = 0·62*; II and V a = −0·63*, p = −0·52; I and III a = 0·56, p = 0·50; IV and VI a = 0·82*, p = 0·63; III and VI a = 0·18, p = −0·03.

intensity response of the DA neurons resembled that observed after preoptic stimulation with regard to prolactin: No significant correlation in the absence of atropine, appearance of a positive correlation between *LH* and intensity in atropine-treated rats. This time, it was the *LH* level that rose in atropine-treated stimulated animals On the other hand, fluorescence intensity and *prolactin* levels showed a similar positive relationship with or without atropine. The type of response observed after preoptic stimulation was relatively isolated, as the changes induced by stimulation of the nuc. of diagonal tract, bed nucleus of stria terminalis and midbrain VMT rather resembled the amygdala-type. A certain analogy to the contrasting effects of atropine may be found in the action of nicotine which was recently reported to reduce *LH* as well as prolactin surges, the effect on the latter hormone depending upon the procedure used to elicit the surge (BLAKE *et al.*, 1972; BLAKE and SAWYER, 1972).

The rather complex results do not allow to design a generally applicable scheme with regard to the response of the DA cell group and hormonal changes. There may be several reasons for that: (1) It may well be that the DA neuron population is *inhomogeneous* with regard to function (cf. LICHTENSTEIGER, 1969b; BJÖRKLUND, *et al.*, 1973). Our results suggest an activation of DA neurons located predominantly in the anterior part in connection with *LH* release (prolactin inhibition?) on one hand and a relation between increase in prolactin levels and activation of DA neurons with a more uniform distribution throughout the region, on the other hand. The latter phenomenon did probably not result from a direct feedback action of prolactin such as has been described for different conditions (HÖKFELT and FUXE, 1972), since we did not obtain a general positive correlation between intensities and the levels of this hormone in all experimental groups. (2) Stimulation in different sites most probably elicits *different additional effects* on releasing-factor release, either through synapses at the releasing factor neurons or through effects at the level of the median eminence. In this context, noradrenergic and serotoninergic projections may be considered (KALRA and MCCANN, 1972; KORDON, 1969) but also eventually stimulation of releasing factor neurons, even in the preoptic area (cf KELLER and LICHTENSTEIGER, 1971). (3) It is possible that hormone levels sometimes did not change because the magnitude of the response of the DA neurons did not reach the necessary *threshold.* However, this cannot be the only reason for the observed discrepancies because intensity changes of similar magnitude were accompanied by different effects on hormone levels.

In conclusion, it appears that influences from a variety of extrahypothalamic sites, notably from *limbic structures* and *ascending brainstem systems*, reach the tubero-infundibular DA neurons. The transmission of such influences seems to depend in part upon the activity of *cholinergic systems.* The indications for a rather complex response pattern and a possible inhomogeneity of the DA neuron group may eventually help to reconcile the divergent conclusions that have been reached with regard to facilitation or inhibition of *LH* and prolactin secretion in various laboratories, including our own, especially if in addition, the possible existence of NA neurons at the level of the external layer of the median eminence (BJÖRKLUND *et al.*, 1970) is considered (cf. AHRÉN *et al.*, 1971; DONOSO *et al.*, 1971; FUXE *et al.*, 1967, 1969, 1972; KAMBERI *et al.*, 1969, 1971; KORDON and GLOWINSKI, 1969; KORDON, 1971; SCHNEIDER and MCCANN, 1970; Van MAANEN and SMELIK, 1968, WUTTKE *et al.*, 1971).

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REFERENCES

AHRÉN K., FUXE K., HAMBERGER L. and HÖKFELT T. (1971) *Endocrinology* **88,** 1415–1424.
BJÖRKLUND A., HROMEK F., OWMAN C. and WEST K. A. (1970) *Brain Res.* **17,** 1–23.
BJÖRKLUND A., MOORE R. Y., NOBIN A. and STENEVI U. (1973) *Brain Res.* **51,** 171–191.
BLAKE C. A. and SAWYER C. H. (1972) *Science* **177,** 619–621.
BLAKE C. A., SCARAMUZZI R. J., REID L. N., KANEMATSU S. and SAWYER C. H. (1972) *Endocrinology* **91,** 1253–1258.
DONOSO A. O., BISHOP W., FAWCETT C. P., KRULICH L. and MCCANN S. M. (1971) *Endocrinology* **89,** 774–784.
FUXE K., HÖKFELT T. and NILSSON O. (1967) *Life Sci.* **6,** 2057–2061.
FUXE K., HÖKFELT T. and NILSSON O. (1969) *Neuroendocrinology* **5,** 107–120.
FUXE K., HÖKFELT T., SUNDSTEDT C.-D., AHRÉN K. and HAMBERGER L. (1972) *Neuroendocrinology* **10,** 282–300.
HEINRICH U., LICHTENSTEIGER W. and LANGEMANN H. (1971) *J. Pharmacol. Exp. Ther.* **179,** 259–267.
HÖKFELT T. and FUXE K. (1972) *Neuroendocrinology* **9,** 100–122.
KALRA S. P. and MCCANN S. M. (1972) *IV Int. Congr. Endocrinology*, Abstract no. 508, Excerpta Medica Intern. Congr. Series no. 256.
KAMBERI I. A., MICAL R. S. and PORTER J. C. (1969) *Science* **166,** 388–389.
KAMBERI I. A., MICAL R. S. and PORTER J. C. (1971) *Endocrinology* **88,** 1012–1020.
KEHR W., CARLSSON A. and LINDQVIST M. (1972) *Arch. Pharmacol.* **274,** 273–280.
KELLER P. J. and LICHTENSTEIGER W. (1971) *J. Physiol.* **219,** 385–401.
KORDON C. (1969) *Neuroendocrinology* **4,** 129–138.
KORDON C. (1971) *Neuroendocrinology* **7,** 202–209.
KORDON C. and GLOWINSKI J. (1969) *Endocrinology* **85,** 924–931.
LICHTENSTEIGER W. (1969a) *J. Pharmacol. Exp. Ther.* **165,** 204–215.
LICHTENSTEIGER W. (1969b) *J. Physiol.* **203,** 675–687.
LICHTENSTEIGER W. (1970) *Prog. Histochem. Cytochem.* **1,** 185–276.
LICHTENSTEIGER W. (1971) *J. Physiol.* **218,** 63–84.
LICHTENSTEIGER W. (1973) *Endocrinology, Proc. IV Intern. Congress*, Excerpta Medica, Amsterdam, in press.
LIENHART R. and LICHTENSTEIGER W. (1973) *Arch. Pharmacol.* **277,** (suppl), R43.
SCHLUMPF M. (1973) Analytische Mikromethode zur fluorimetrischen Bestimmung von Monoaminen. Thesis, Eidg. Technische Hochschule, Zürich.
SCHNEIDER H. P. G. and MCCANN S. M. (1970) *Endocrinology* **87,** 249–253.
VAN MAANEN J. H. and SMELIK P. G. (1968) *Neuroendocrinology* **3,** 177–186.
WUTTKE W., CASSELL E. and MEITES J. (1971) *Endocrinology* **88,** 737–741.