HORMONE RECEPTORS IN THE BRAIN

HORMONE RECEPTORS IN THE BRAIN

Papers by
Junzo Kato, Richard E. Whalen, Richard Maurer, Richard P. Michael, Walter E. Stumpf, Conwell H. Anderson, Arne Attramadal, D.E. Woolley, J. Presl, Angelo C. Notides, W.W. Leavitt, Bruce McEwen, J.A. Resko, Joseph Altman, R.T. Lobl et al.

MSS Information Corporation
655 Madison Avenue, New York, N.Y. 10021

Library of Congress Cataloging in Publication Data
Main entry under title:

Hormone receptors in the brain.

1. Brain--Addresses, essays, lectures.
2. Hormones--Addresses, essays, lectures.
I. Kato, Junzo. [DNLM: 1. Brain--Drug effects
--Collected works. 2. Brain chemistry--Collected
works. 3. Hormones--Metabolism--Collected works.
4. Receptors, Drug--Collected works. WL 102 H812 1973]
QP376.H75 1973 599'.3233 72-13551
ISBN 0-8422-7078-7

TABLE OF CONTENTS

HORMONE RECEPTORS IN THE BRAIN

Papers by
Junzo Kato, Richard E. Whalen, Richard Maurer, Richard P. Michael, Walter E. Stumpf, Conwell H. Anderson, Arne Attramadal, D.E. Woolley, J. Presl, Angelo C. Notides, W.W. Leavitt, Bruce McEwen, J.A. Resko, Joseph Altman, R.T. Lobl et al.

MSS Information Corporation
655 Madison Avenue, New York, N.Y. 10021

Library of Congress Cataloging in Publication Data
Main entry under title:

Hormone receptors in the brain.

1. Brain--Addresses, essays, lectures.
2. Hormones--Addresses, essays, lectures.
I. Kato, Junzo. [DNLM: 1. Brain--Drug effects
--Collected works. 2. Brain chemistry--Collected
works. 3. Hormones--Metabolism--Collected works.
4. Receptors, Drug--Collected works. WL 102 H812 1973]
QP376.H75 1973 599'.3233 72-13551
ISBN 0-8422-7078-7

TABLE OF CONTENTS

CREDITS AND ACKNOWLEDGEMENTS

Altman, Joseph; and Gopal D. Das, "Autoradiographic and Histological Evidence of Postnatal Hippocampal Neurogenesis in Rats," *Journal of Comparative Neurology*, 1965, 124:319-335.

Anderson, Conwell H.; and Gilbert S. Greenwald, "Autoradiographic Analysis of Estradiol Uptake in the Brain and Pituitary of the Female Rat," *Endocrinology*, 1969, 85:1160-1165.

Attramadal, Arne, "Cellular Localization of ^{3}H-Oestradiol in the Hypothalamus: An Autoradiographic Study in Male and Female Rats," *Zeitschrift für Zellforschung*, 1970, 104:572-581.

Attramadal, Arne; and Asbjörn Aakvaag, "The Uptake of ^{3}H-Oestradiol by the Anterior Hypophysis and Hypothalamus of Male and Female Rats," *Zeitschrift für Zellforschung*, 1970, 104:582-596.

Attramadal, Arne, "Cellular Localization of ^{3}H-Oestradiol in the Hypophysis: An Autoradiographic Study in Male and Female Rats," *Zeitschrift für Zellforschung*, 1970, 104:597-614.

Kato, Junzo; and Claude A. Villee, "Factors Affecting Uptake of Estradiol-6,7- ^{3}H by the Hypophysis and Hypothalamus," *Endocrinology*, 1967, 80:1133-1138.

Kato, Junzo, "*In Vitro* Uptake of Tritiated Oestradiol by the Anterior Hypothalamus and Hypophysis of the Rat," *Acta Endocrinologica*, 1970, 64:687-695.

Kato, Junzo, "*In Vitro* Uptake of Tritiated Oestradiol by the Rat Anterior Hypothalamus During the Oestrous Cycle," *Acta Endocrinologica*, 1970, 63:577-584.

Kato, Junzo; Takashi Kobayashi; and Claude A. Villee, "Effect of Clomiphene on the Uptake of Estradiol by the Anterior Hypothalamus and Hypophysis," *Endocrinology*, 1968, 82:1049-1052.

Kato, J., "Estrogen Receptors in the Hypothalamus and Hypophysis in Relation to Reproduction," *Excerpta Medica: Hormonal Steroids*, 1970, No. 219:764-773.

Kato, Junzo; Yachiyo Atsumi; and Machiko Inaba, "Development of Estrogen Receptors in the Rat Hypothalamus," *Journal of Biochemistry*, 1971, 70:1051-1053.

Kato, Junzo; Yachiyo Atsumi; and Masami Muramatsu, "Nuclear Estradiol Receptor in Rat Anterior Hypophysis," *Journal of Biochemistry*, 1970, 67:871-872.

Kato, Junzo; Yachiyo Atsumi; and Machiko Inaba, "A Soluble Receptor for Estradiol in Rat Anterior Hypophysis," *Journal of Biochemistry*, 1970, 68:759-761.

Leavitt, W.W.; J.P. Friend; and J.A. Robinson, "Estradiol: Specific Binding by Pituitary Nuclear Faction *in Vitro*, " *Science*, 1969, 165:496-498.

Lobl, R.T.; and S. Schapiro, "Alterations in the Binding of ^{3}H-Testosterone During Development in the Rat," *Neuroendocrinology*, 1971, 8:107-115.

Maurer, Richard; and Dorothy Woolley, "Distribution of ^{3}H-Estradiol in Clomiphene-Treated and Neonatally Androgenized Rats," *Endocrinology*, 1971, 88:1281-1287.

McEwen, Bruce S.; and Donald W. Pfaff, "Factors Influencing Sex Hormone Uptake by Rat Brain Regions. I. Effects of Neonatal Treatment, Hypophysectomy, and Competing Steroid on Estradiol Uptake," *Brain Research*, 1970, 21:1-16.

McEwen, B.S.; D.W. Pfaff; and R.E. Zigmond, "Factors Influencing Sex Hormone Uptake by Rat Brain Regions. II. Effects of Neonatal Treatment and Hypophysectomy on Testosterone Uptake," *Brain Research*, 1970, 21:17-28.

Michael, Richard P., "Estrogen-Sensitive Neurons and Sexual Behavior in Female Cats," *Science*, 1962, 136:322-323.

Notides, Angelo C., "Binding Affinity and Specificity of the Estrogen Receptor of the Rat Uterus and Anterior Pituitary," *Endocrinology*, 1970, 87:987-992.

Presl, J.; S. Röhling; J. Horský; and J. Herzmann, "Changes in Uptake of ^{3}H-Estradiol by the Female Rat Brain and Pituitary from Birth to Sexual Maturity," *Endocrinology*, 1970, 86:899-902.

Resko, J.A.; R.W. Goy; and C.H. Phoenix, "Uptake and Distribution of Exogenous Testosterone-1,2- ^{3}H in Neural and Genital Tissues of the Castrate Guinea Pig," *Endocrinology*, 1967, 80:490-498.

Stumpf, Walter E., "Estrogen-Neurons and Estrogen-Neuron Systems in the Periventricular Brain," *The American Journal of Anatomy*, 1970, 129:207-217.

Stumpf, Walter E., "Subcellular Distribution of ^{3}H-Estradiol in Rat Uterus by Quantitative Autoradiography: A Comparison Between ^{3}H-Estradiol and ^{3}H-Norethynodrel," *Endocrinology*, 1968, 83:777-782.

Stumpf, Walter E., "Estradiol-Concentrating Neurons: Topography in the Hypothalamus by Dry-Mount Autoradiography," *Science*, 1968, 162:1001-1003.

Stumpf, Walter E.; and Madhabananda Sar, "Estradiol Concentrating Neurons in the Amygdala," *Proceedings of the Society for Experimental Biology and Medicine*, 1971, 136:102-106.

Stumpf, Walter E., "Probable Sites for Estrogen Receptors in Brain and Pituitary," *Journal of Neuro-Visceral Relations*, 1971, X:51-64.

Whalen, Richard E.; and William G. Luttge, "Long-Term Retention of Tritiated Estradiol in Brain and Peripheral Tissues of Male and Female Rats," *Neuroendocrinology*, 1970, 6:255-263.

Whoolley, D.E.; C.F. Holinka; and P.S. Timiras, "Changes in ^{3}H-Estradiol Distribution with Development in the Rat," *Endocrinology*, 1969, 84:157-161.

PREFACE

The role of gonadal hormones in the sexual differentiation of the hypothalamus and in regulation of sexual behavior is well established. During the past fifty years, many examples have been described which show a *direct* interaction of gonadal steroids with the brain in the regulation of pituitary gonadotrophin secretion and the stimulation of sex-specific behavior. However, the nature of the interaction of gonadal hormones with the brain has only recently been studied. As documented in this volume there is now ample evidence of receptor molecules for steroid hormones in the brain.

The early work of Glascock, Hoekstra, and others has demonstrated the existence of cytoplasmic and nuclear steroid receptor proteins which stereospecifically bind estradiol in the mammalian uterus. Studies are in progress which should elucidate the properties of these potential estrogen receptors, their interactions with steroid hormones and the cell nucleus, and the role of their interactions in the uterotrophic effects of estradiol. Several studies on uterine receptors are included in this collection, because work in this tissue has provided such an important model in the search for sex hormone receptors in the brain.

Investigations of brain binding sites for estradiol have been among the most fruitful efforts in the rapidly growing field of neuroendocrinology. The following papers illustrate various approaches to date. Tissue uptake studies (involving measurement of incorporated radioactivity in various brain regions following injection of tritiated estradiol) by Eisenfeld, Axelrod, and others have pointed to the presence of limited capacity, stereospecific and regionally specific binding sites for estradiol in the female rat brain. Autoradiographic results of Michael, Pfaff, and others have generally paralleled the biochemical findings for cat as well as rat. Further studies by Zigmond and McEwen showed that a major percentage of incorporated estradiol is bound and retained by cell nuclei of the hypothalamus and preoptic area. Other investigations have also revealed that soluble, cytoplasmic macromolecules of hypothalamus and anterior pituitary stereospecifically bind estradiol *in vivo* and *in vitro*, forming a slowly dissociating complex, similar to the estradiol-cytoplasmic protein complex in the uterus. This complex can be isolated by chromatography and by density gradient centrifugation. Although the specific cellular functions of the estradiol binding sites in the brain is unknown at present, evidence found in these papers strongly indicates a direct role of the

hormone binding sites to regions responsive to implants of estrogens, extreme specificity of binding for active estrogens, and finally presence of high affinity of binding sites in cell nuclei.

In contrast with these findings for estradiol, there is little information about brain receptors for testosterone. McEwen *et al.* and Resko observed that *in vivo* uptake of tritiated testosterone by the adult brain had a distribution in the brain similar to patterns obtained with labelled estradiol. Autoradiographic studies of Pfaff also demonstrated selective retention of testosterone by limbic and hypothalamic structures in the rat. The recent work of Zigmond *et al.*, with ring doves indicates concentration of testosterone by cell nuclei in the hypothalamus.

Taken together, the studies in this volume provide firm evidence that steroid hormone dependent control mechanisms in the brain involve interaction of hormones with the cell nucleus, as is the case in many other tissues. The role of variable nuclear gene activity in regulating sex differences in behavior as well as other neural phenomena remains to be determined.

Linda Plapinger
January, 1973

Estrogen

Factors Affecting Uptake of Estradiol-6,7-^{3}H by the Hypophysis and Hypothalamus

JUNZO KATO AND CLAUDE A. VILLEE

PREVIOUS experiments in this laboratory (1) had demonstrated the preferential uptake of estradiol by the anterior hypothalamus based upon its accumulation and retention with little or no metabolic conversion. These findings confirmed and extended the reports of accumulation of estradiol in the hypothalamus (2) and suggested the presence in the anterior hypothalamus of specific binding sites for estradiol similar to those in the uterus and vagina found by other investigators (2–6). The specificity of the action of estradiol in those tissues has been attributed to these specific binding sites (6) and a relationship between the uptake of estradiol and estrogenic activity has been reported by Terenius (7).

The present study was undertaken to define further the capacity and structural specificity of the sites for the binding of estradiol in the anterior hypothalamus and the anterior hypophysis.

Materials and Methods

Adult ovariectomized rats weighing 230–250 g supplied by the Charles River Breeding Laboratory, Wilmington, Mass., were used 8 or 9 days after the operation. Vaginal smears were examined to establish diestrus. Rats were examined after sacrifice to make sure that ovariectomy had been complete.

The animals were fasted 12 hr, then injected intraperitoneally with 0.3 μg of 17β-estradiol-^{3}H with or without added unlabeled 17β-estradiol in 0.5 ml of physiological saline containing 10% ethanol. The animals were killed by decapitation 1 hr later.

Dissection of the Brain. The entire brain was removed as rapidly as possible and freed of blood vessels. The blood vessels surrounding the hypothalamic area on the base of the brain were dissected away and blood adhering to the surface of the hypothalamus was blotted with filter paper. The entire hypothalamus was cut out as a block limited anteriorly by the optic chiasma, laterally by the hypothalamic fissures, and posteriorly by the mammillary body. The block was 2 ml deep from the basal surface of the hypothalamus. The median eminence was resected, the hypothalamus was divided into anterior, middle and posterior sections as described previously (1), and the tissues were weighed on an analytical balance. The remaining parts of the brain were washed several times

TABLE 1. Distribution of radioactivity in rat brain and hypophysis after the injection of tritiated estradiol with increasing amounts of nonradioactive estradiol

Tissue	Total estradiol injected, μg				
	0.3	0.9	1.8	4.0	8.0
Anterior hypothalamus	72.9 ±9.4 (5)	54.5 ±4.3 (6)	46.8 ±3.4 (6)††	30.9 ±1.7 (5)††††	21.0 ±2.3 (5)††††
Middle hypothalamus	40.9 ±3.1 (5)	45.1 ±6.2 (6)	35.5 ±5.7 (6)	23.0 ±2.8 (5)††††	17.3 ±2.0 (5)††††
Posterior hypothalamus	32.6 ±1.1 (5)	36.3 ±3.7 (5)	31.2 ±3.4 (6)	21.9 ±3.4 (5)††	17.3 ±2.6 (5)††††
Median eminence	117.2	128.0	116.1	44.8	31.2
Anterior hypophysis	472.2 ±89.4 (5)	456.1 ±72.4 (6)	376.9 ±36.3 (6)	249.2 ±13.9 (5)†	136.8 ±5.9 (5)†††
Posterior hypophysis	43.1	66.1	62.2	21.6	19.6
Cerebral cortex	17.6 ±2.3 (5)	17.9 ±2.8 (6)	16.2 ±1.7 (6)	13.3 ±2.3 (5)	11.9 ±1.7 (5)†††
Cerebellum	20.4 ±1.7 (5)	22.4 ±3.4 (6)	19.3 ±2.3 (6)	16.5 ±3.1 (5)	13.3 ±1.7 (5)

0.3 μg of estradiol-6,7-^{3}H (specific activity 42 c/mmole) was injected intraperitoneally into adult ovariectomized rats together with increasing amounts of nonradioactive estradiol. The animals were killed 1 hr after the injection. The values given in the table represent mean dpm/mg wet weight ± standard error. The number of animals in the experimental group is given in parentheses. The values for median eminence and posterior hypophysis were obtained from pooled tissues.

† $p < .05$; †† $p < .025$; ††† $p < .01$; and †††† $p < .005$, compared with the respective control values of the first column.

in ice cold physiological saline and blotted with filter paper. Cortical tissue was excised from the frontal and parietal lobes of the brain, and the left half of the cerebellum was taken for analysis. The anterior and posterior hypophyses were separated. The median eminence from 2 or 3 rats and the posterior hypophyses from 2 or 3 rats were pooled for extraction.

Extraction of Radioactivity. Each tissue was homogenized in 5 ml of ethanol:acetone 1:1 (v/v) in a glass homogenizer and extracted 5 times with 5 ml of the same solvent. The extracts were pooled and dried in scintillation vials at temperatures kept below 55 C.

Chemicals. All solvents were redistilled before use. Estradiol-6,7-^{3}H (specific activity 42 c/mmole) was obtained from New England Nuclear Corporation and tested for impurities by thin-layer chromotagraphy in 3 different systems. 17β-Estradiol was supplied by Sigma Chemical and 17α-estradiol (melting point 221–223 C) was supplied by Mann Research Laboratories, New York.

Measurement of Radioactivity. Tritium was measured in a Packard Model 3324 Scintillation Counter using as scintillation fluid a solution containing 4 g of 2,5-diphenyloxazole and 0.04 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl) benzene in 1 liter of toluene ethanol 98:2(v/v). Enough counts were accumulated to reduce the probable error of counting to less than 1%. No significant quenching occurred in these samples as determined by the use of internal tritiated toluene standards. The radioactivity remaining in the residue of the brain and hypophysis was less than 1% of the initial radioactivity of the tissue.

Results

To determine whether the tissues of the brain and hypophysis have a limited capacity for the uptake of estradiol, 0.3 μg of tritiated estradiol was injected intraperitoneally together with unlabeled estradiol to give a total of 0.3, 0.9, 1.8, 4.0, or 8.0 μg. One hour after injection the animals were killed and tissues were removed for the measurement of radioactivity. No significant difference was found in the concentration of radioactivity in the cerebral cortex at any of these doses; in contrast, amounts of estradiol greater than 1.8 μg depressed the amount of radioactivity in the anterior hypothalamus (Table 1). A dose of 4 μg or more decreased the uptake of labeled estradiol in other parts of the hypothalamus and the anterior hypophysis. The uptake of estradiol in the median

eminence and posterior hypophysis was slightly depressed when the amount of estradiol injected exceeded 4 μg; the uptake in the cerebellum was depressed only when the dosage exceeded 8 μg.

From the amount of radioactivity taken up by each tissue and the specific activity of the estradiol administered, it was possible to estimate the total amount of estradiol taken up per mg of tissue at each dose. This was expressed as a ratio of the amount taken up at the lowest dosage (0.3 μg), and plotted (Fig. 1) as a function of the total dose of estradiol administered. The amount of estradiol taken up by the anterior hypothalamus and anterior hypophysis reached a plateau within the range of estradiol doses administered in these experiments. In contrast, the amount taken up by the cerebral cortex and cerebellum showed a simple dose dependent increase in the amount taken up over the entire range. These results indicate a limited capacity for the binding of estradiol at receptor sites in the anterior hypothalamus and anterior hypophysis but no such limited capacity in the cerebellum or cerebral cortex.

In another series of experiments, rats were injected subcutaneously with varying amounts of unlabeled estradiol three hours before the injection of 0.3 μg of tritiated estradiol. The tritiated estradiol was injected intraperitoneally, the animals were killed one hour later, and the concentration of radioactivity in the brain and pituitary was measured (Table 2). Pretreatment with 0.1 μg of estradiol had no significant effect on the uptake of estradiol by the anterior or posterior hypothalamus but did decrease the uptake in the middle hypothalamus. Pretreatment with 1 μg or more of unlabeled estradiol greatly decreased the concentration of radioactivity in all parts of the hypothalamus. The uptake of estradiol by the anterior hypophysis, like that of the anterior hypothalamus, was decreased by pretreatment with 1 μg or more of unlabeled estradiol. In these tissues increasing doses of estradiol led to a greater

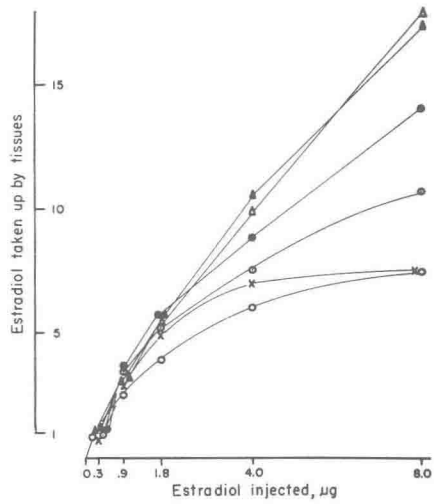

FIG. 1. Uptake of estradiol by regions of the brain and hypophysis as a function of the amount of estradiol injected. Adult ovariectomized rats were injected with 0.3 μg of 17β-estradiol-6,7-^{3}H, specific activity 42 c/mmole, plus varying amounts of unlabeled estradiol, and were killed 1 hr later. The amount of estradiol taken up by the tissue was calculated from its content of radioactive estradiol and the specific activity of the estradiol administered. The amount of estradiol taken up when 0.3 μg of estradiol was injected was taken as unity and the amounts taken up when larger amounts were injected are expressed in relation to this value, so that the several tissues may be compared on the same scale. ○ = Anterior hypothalamus; ⊙ = middle hypothalamus; ● = posterior hypothalamus; x = anterior hypophysis; △ = cerebral cortex; ▲ = cerebellum.

depression in the concentration of radioactivity.

A third series of experiments attempted to determine whether estradiol taken up by the hypothalamus and hypophysis could be removed by a second injection of nonradioactive estradiol. Ten μg of estradiol dissolved in 0.5 ml of 10% ethanol-saline was injected intraperitoneally into rats 30 minutes after the intraperitoneal administration of tritiated estradiol. Rats were killed 30 minutes after the last injection and tissues were dissected out for