Estrogen-2-hydroxylase in the Brain of the Male African Catfish, *Clarias gariepinus*

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Estrogen-2-hydroxylase activity, involved in the biosynthesis of catecholestrogens, was localized in the brain of the male African catfish, *Clarias gariepinus*, by means of a radiometric assay using [2-³H]estradiol as substrate. Fore- and midbrain were divided in 18, 500-µm thick, transverse sections from which small defined areas were punched out and assayed. The estrogen-2-hydroxylase activity was calculated from the release of tritium during hydroxylation, and expressed in femtomole catecholestradiol · milligram^{-1} tissue · hour^{-1}. The enzyme could be demonstrated throughout the brain. A high activity (>350 fmol) was observed in the telencephalon, in particularly the rostral part and the area ventralis pars dorsalis; in the diencephalon in the preoptic region, including the magnocellular part of the preoptic nucleus and the rostral part of the anterior periventricular nucleus; and in the area tuberalis, including the nucleus lateralis tuberis, the rostral part of the nucleus anterior tuberis, the caudal part of the nucleus posterior periventricularis, and in the nucleus recessus posterioris. Also a high activity was detected in the mesencephalic tectum opticum and the dorsolateral part of the torus semicircularis. The ventral mesencephalon showed a moderate (200–350 fmol) to low (<200 fmol) activity, whereas the lowest activity was found in the hindbrain (118 fmol). The significance of the biosynthesis of catecholestrogens in the brain is discussed in light of the negative feedback mechanism of gonadal steroids on gonadotropin release. © 1988 Academic Press, Inc.

In the brain of mammals, aromatization of androgens to estrogens appears to be an essential step in the regulation of several neuroendocrine and behavioral functions (Naftolin *et al.*, 1975b; McEwen *et al.*, 1979, 1982). The brain of the male African catfish, *Clarias gariepinus,* can also aromatize androgens to estrogens (Timmers *et al.,* 1987).

Estrogens, in their turn, can be metabolized to catecholestrogens, in particular 2-hydroxyestrogens. This has been demonstrated in many mammalian tissues, including brain and pituitary gland (for reviews: Fishman, 1976, 1981; Ball and Knuppen, 1980; Barnea *et al.,* 1984). So far, in teleosts estrogen-2-hydroxylase, the enzyme

responsible for the formation of the 2-hydroxyestrogens (catecholestrogens), has been detected only in the brain of the rainbow trout, *Salmo gairdneri* (Lambert and Van Oordt, 1982), and the African catfish, *Clarias gariepinus* (Lambert *et al.,* 1987).

Catecholestrogens may be considered as potential mediators of estrogen action, particularly in the brain and pituitary gland (MacLusky *et al.,* 1981). This includes the negative feedback effect of estrogens on gonadotropin (GTH) release (Knuppen, 1981; Breuer *et al.,* 1981).

In teleosts no data are available about the effects of catecholestrogens on GTH release. It is known, however, that reduction of the GTH level can be induced by estrogens and aromatizable androgens and not by nonaromatizable androgens (Billard,

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1978, De Leeuw et al., 1986). Thus, it seems that of the steroids only the estrogens, or their metabolites (catecholestrogens), are directly involved in regulating GTH release. The mechanism of action of estrogens on GTH regulation might be the influence of catecholestrogens on the metabolism of catechol structures. Indeed, it is known that in teleosts, including the goldfish, Carassius auratus (Chang and Peter, 1983), and the African catfish (De Leeuw et al., 1985), catecholamines, especially dopamine, inhibit the GTH release. A biochemical link between catecholestrogens and catecholamines might be accomplished by the enzyme catechol-O-methyltransferase (COMT), as suggested by Lloyd et al. (1978), Knuppen (1981), and Breuer et al. (1981). Starting from this suggestion the following feedback model (see also Fig. 1) has been postulated by Lambert et al. (1984) (see also Goos et al., 1985b; De Leeuw et al., 1986; Goos, 1987; Timmers and Lambert, 1988).

According to this feedback model, in specific brain centers androgens will be aromatized to estrogens. These estrogens, in their turn, will be hydroxylated to catecholestrogens, which subsequently are methylated by COMT. This enzyme is also responsible for the methylation of dopamine. If there is a competitive interaction between both catechol substrates and the enzyme COMT, then the presence of catecholestrogens might inhibit the inactivation of dopamine, resulting in a stimulation of the dopaminergic inhibition of the GTH release.

This hypothesis presupposes the presence of the enzymes involved in the conversion of androgens into methylated catecholestrogens (methoxyestrogens) in catfish brain, preferably in centers regulating GTH release. In the brain of the African catfish the enzyme aromatase has been studied before, including its characterization (Timmers and Lambert, 1987) and localization (Timmers et al., 1987).
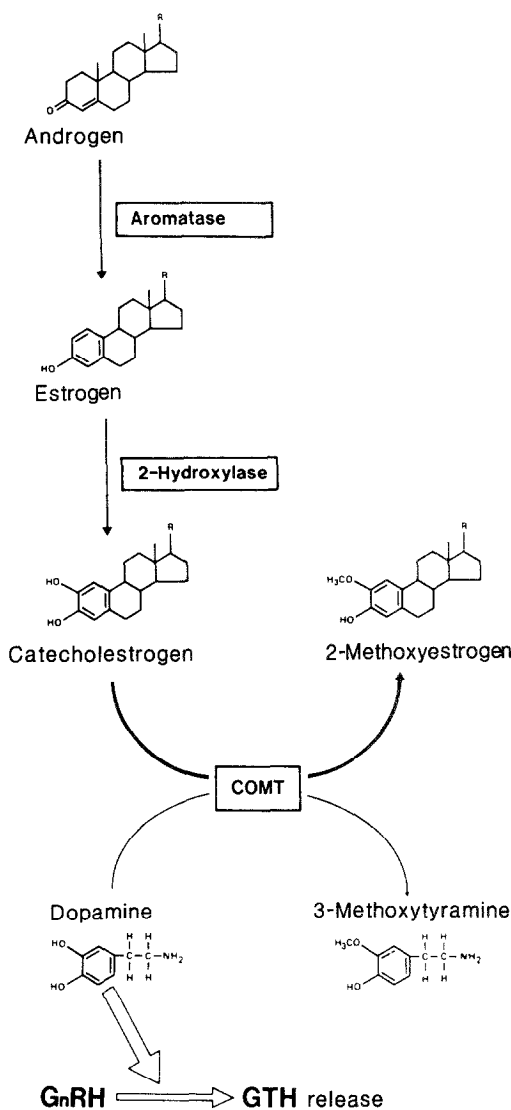

FIG. 1. Steroid feedback model representing the interaction between sex steroids and dopamine in the regulation of GTH release.

This study discusses the localization of the enzyme estrogen-2-hydroxylase. A combined microanatomical and biochemical technique was applied. Using [2-^{3}H]-estradiol as substrate, estrogen-2-hydroxylase activity was determined in small pieces of tissue (0.3 mg) punched out of defined areas of the brain.

MATERIALS AND METHODS

Chemicals

[2-³H]Estradiol (sp act 22.1 Ci/mmol) was purchased from Amersham, and before use purity was checked by thin-layer chromatography. Reference and carrier steroids were obtained from Merck A.G., and the cofactor NADPH and the buffer Hepes were from Boehringer. All chemicals and solvents (Baker) were of analytical grade.

Animals

African catfish, *C. gariepinus*, were reared from eggs to maturity in the laboratory. The breeding techniques and hatchery conditions have been reported by De Leeuw *et al.* (1985). Six mature males 10–12 months of age, weighing about 500 g, were used. Under the hatchery conditions the testes of these animals contained ripe sperm, as fertilization tests confirmed. After decapitation the brains were removed, immediately frozen on dry ice, and stored at −70° until use.

Microdissection of the Brain

Sectioning of the brain was carried out with a Lancer vibratome at −7°, as reported by Timmers *et al.* (1987). From each brain 18 transverse slices (500 μm) were cut, beginning at the rostral tip of the telencephalon (Fig. 2). The slices were collected on glass slides and transferred to a cold plate (−10°). With a cold hollow needle, inner diameter 1 mm, specific areas of the brain could be punched out (Palkovits and Brownstein, 1983). These punches (average weight 0.33 mg) were prepared for the estrogen-2-hydroxylase assay. Technically, it was not always possible to obtain punches at exactly the same location from comparable sections of the six animals. Only the comparable punches of each animal were used in the assay. The sections and punches were identical with those given in a previous study (Timmers *et al.*, 1987) concerning aromatase activity in the brain. This offers the possibility of comparing the activity of the enzymes aromatase and estrogen-2-hydroxylase.

Estrogen-2-hydroxylase Assay

The estrogen-2-hydroxylase assay, using [2-³H]-estradiol as substrate, is based on the enzymatically induced release of the tritium label during the conversion of estradiol into 2-hydroxyestradiol (catecholestradiol). The 2-hydroxylase activity can be deduced from the amount of catecholestradiol synthesized per incubation. This amount can be established from the tritium released into the medium (Numazawa *et al.*, 1980). The enzyme activity is expressed in femtomole catecholestradiol per milligram tissue per hour. Per incubation two symmetrical punches were taken; one from the left and one from the right side of the brain section. These punches were transferred to a 10-ml centrifuge tube containing [2-³H]estradiol (0.4 μCi, 0.4 μM) dissolved in propylene glycol (final concentration 5%, v/v), the cofactor NADPH (2 mM), and Hepes buffer (0.1 M, pH 7.4). The final incubation volume was 50 μl. The incubations were carried out at 26° in an air atmosphere under continuous shaking. The incubations were terminated after 30 min by placing the tubes in an ice bath. Then the water volume was brought up to 1 ml. In order to remove the remaining nonconverted [2-³H]estradiol from the aqueous medium, an organic extraction was carried out with 4 ml of dichloromethane. Before this extraction 40 μg nonlabeled estradiol was added, as carrier. The amount of tritium label, released into the water fraction, was determined by scintillation counting. To that end an aliquot of 0.5 ml of the water fraction was counted in a Searle Analytic 92 scintillation counter
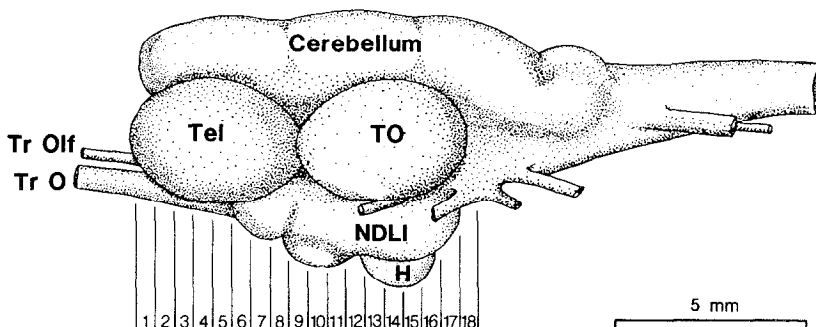

FIG. 2. Lateral view of the brain of the male *Clarias gariepinus*. The fore- and midbrain are divided into 18 500-μm transverse sections numbered from 1 to 18. These numbers correspond to the sections in Fig. 3. For abbreviations see Table 1.

with a hydrophyl scintillation cocktail (Hydroluma, Baker). Corrections for nonenzymatically induced release of tritium were made after determining the tritium release in control experiments with preheated tissue (10 min, 100°).

RESULTS

Photographs of the consecutive brain sections are shown in Fig. 3. At the right-hand side of each picture neuroanatomical areas are indicated, according to the nomenclature of Nieuwenhuys (1963) and Bass (1981a, b). The left-hand side of each picture shows the circular areas punched out of the section. The relative amount of estrogen-2-hydroxylase is represented by the size of the dark segments in the circles, considering that the activity in punch 4 of
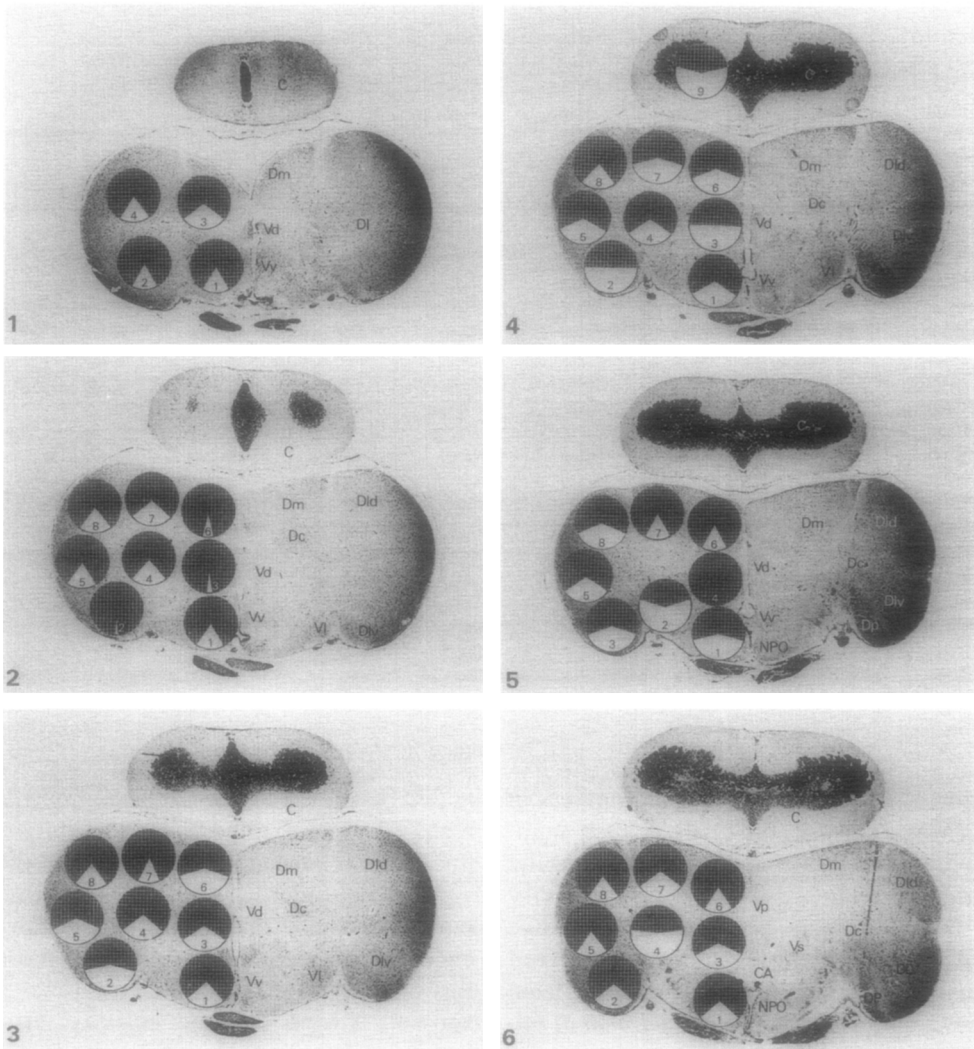

FIG. 3. Eighteen transverse sections of the brain of the male *Clarias gariepinus*, corresponding to the 500-μm sections, as given in Fig. 2. At the right-hand side of each section neuroanatomical areas are given. At the left-hand side the circles (1 mm in diameter) indicate the sites where the punches are taken. The size of the dark segments represents the relative estrogen-2-hydroxylase activity, deduced from the highest activity as found in the ventro–dorsal area of the telencephalon (Vd, section 5, punch 4). For abbreviations see Table 1. Magnification, ×7.
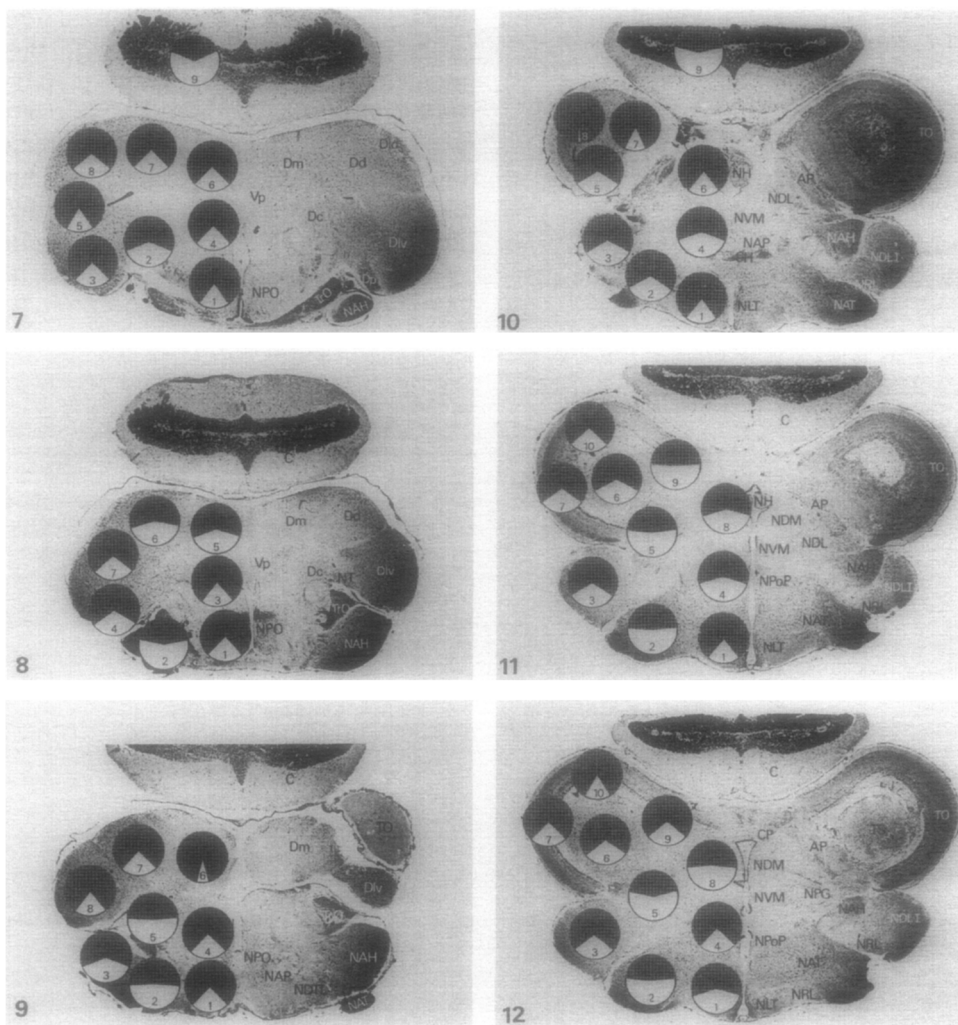
FIG. 3—Continued.

section 5 is fixed at 100%. The numbers in the circles correspond to the punch numbers as given in Table 1. In that table the average estrogen-2-hydroxylase activity per punch area is given as the average amount of catecholestradiol converted from estradiol per hour, calculated from three to six determinations; and also in relative amounts, i.e., in percentage of the highest activity.

Estrogen-2-hydroxylase activity could be demonstrated throughout the brain. For practical reasons the enzyme activity was divided into three arbitrary classes: low

(<200 fmol), moderate (200–350 fmol), and high (>350 fmol). The highest activity (525 fmol) was recorded in the dorsal part of the ventral telencephalon (Vd) (section 5, punch 4). The entire rostral part of the telencephalon (sections 1 and 2) and most of the caudal telencephalon (sections 5–9) showed a high enzyme activity. In the punches of sections 3 and 4 a moderate 2-hydroxylase activity prevailed, but some punches of these sections, including those of the area dorsalis pars lateralis dorsalis (Dld), showed a high activity.

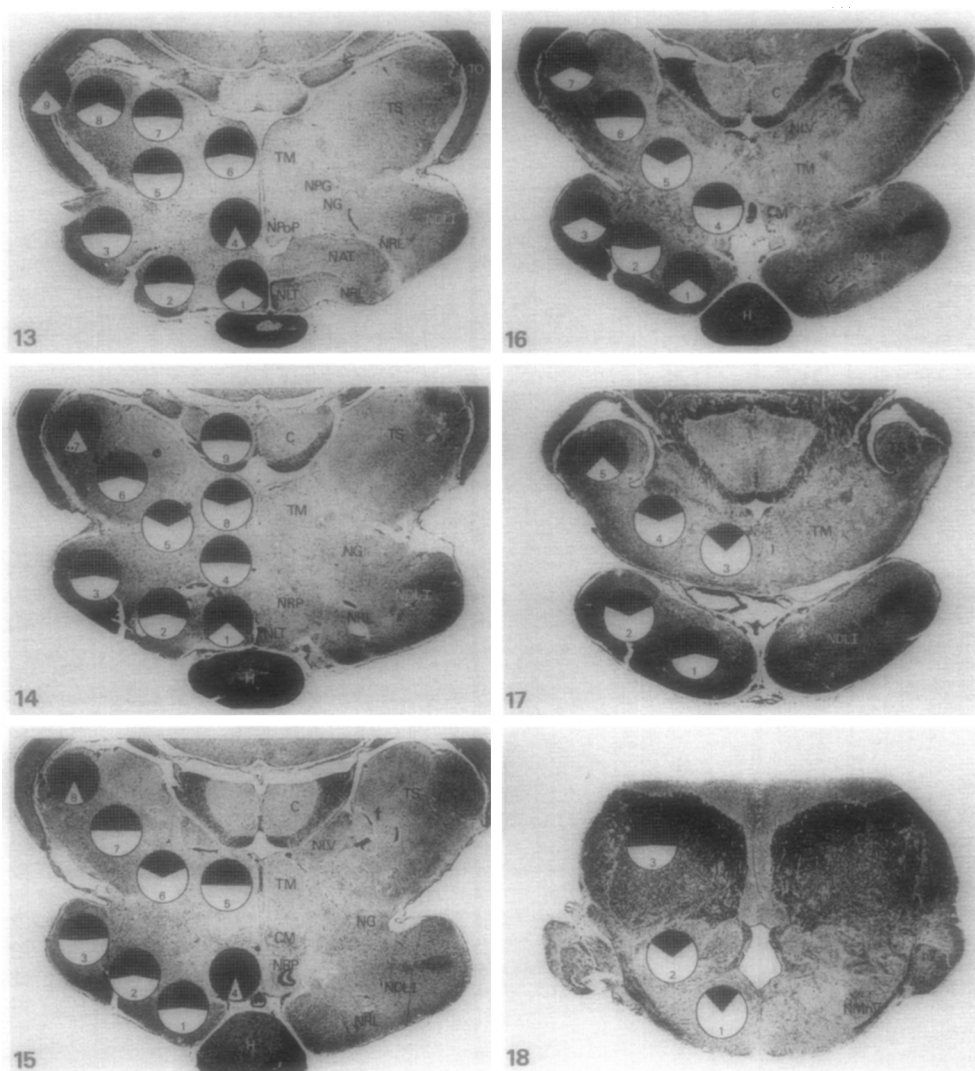In the diencephalon the preoptic region

FIG. 3—*Continued.*

demonstrated a moderate or high estrogen-2-hydroxylase activity. The high activity was measured in the entire magnocellular part of the preoptic nucleus (NPO) and in the rostral part of the anterior periventricular nucleus (NAP) (sections 6–9). In the area tuberalis a moderate enzyme activity prevailed. A high activity was, however, found in the nucleus lateralis tuberis (NLT) (sections 10–14), in the rostral part of the nucleus habenularis (NH) and of the nucleus anterior tuberis (NAT) (section 10), in some areas of the nucleus diffusus lobi inferioris (NDLI) (sections 10–17), the caudal part of the posterior periventricular nucleus (NPoP) (sections 12–13), and in the posterior nucleus recessus (NRP) (sections 14–15).

In the mesencephalon material punched out of the tectum opticum (TO) with few exceptions showed a high enzyme activity (sections 10–13). Also in the dorsolateral part of the torus semicircularis (TS) a high enzyme activity was observed (sections 12–

TABLE 1
QUANTIFICATION OF ESTROGEN-2-HYDROXYLASE ACTIVITY IN LOCALIZED AREAS IN THE BRAIN OF THE
MALE AFRICAN CATFISH, *Clarias gariepinus*

No.[a]	Area[b]	N^c	Amount of catecholestrogen[d]	Prop. distr
1– 1	Tel-Vv	4	437 ± 50	83
2	-Dl	6	439 ± 82	84
3	-Dm	3	375 ± 39	71
4	-Dl	5	426 ± 71	81
2– 1	Tel-Vv	4	432 ± 44	82
2	-Dlv	6	520 ± 86	99
3	-Vd	3	506 ± 81	96
4	-Dld	4	404 ± 58	77
5	-Dld	3	424 ± 71	81
6	-Dm	5	487 ± 92	93
7	-Dld + Dm	5	383 ± 53	73
8	-Dld	5	412 ± 37	79
3– 1	Tel-Vv	6	392 ± 26	75
2	-Dlv	3	303 ± 55	58
3	-Vv + Vd	5	364 ± 74	69
4	-Dc	4	378 ± 91	72
5	-Dld	3	338 ± 25	64
6	-Dm	4	317 ± 57	60
7	-Dm + Dld	6	450 ± 92	86
8	-Dld	6	416 ± 70	79
4– 1	Tel-Vv	6	357 ± 46	68
2	-Dp + Dlv	5	260 ± 47	50
3	-Vv + Vd	3	268 ± 17	51
4	-Dc	3	361 ± 16	69
5	-Dld	3	342 ± 42	65
6	-Dm	3	325 ± 40	62
7	-Dm + Dld	4	312 ± 32	59
8	-Dld	5	409 ± 55	78
9	Met-C	5	227 ± 51	43
5– 1	Tel-Vv, Di-NPO	6	302 ± 38	58
2	Tel-Dp + Tr Olf	3	216 ± 13	41
3	Tel-Dp + Dlv	3	318 ± 57	61
4	-Vd	4	525 ± 82	100
5	-Dlv + Dld	4	361 ± 54	69
6	-Dm	6	443 ± 78	84
7	-Dm	5	440 ± 84	84
8	-Dld	4	330 ± 40	63
6– 1	Di-NPO	6	383 ± 67	73
2	Tel-Dp + Dlv	6	385 ± 71	73
3	-Vs	3	340 ± 56	65
4	-Dc	3	244 ± 20	47
5	-Dld + Dlv	3	421 ± 61	80
6	-Dm	4	429 ± 81	82
7	-Dm	3	366 ± 58	70
8	-Dld	4	420 ± 27	80
7– 1	Di-NPO	6	422 ± 66	80
2	-Tr Olf	6	323 ± 58	62
3	Tel-Dp + Dlv	5	393 ± 64	75
4	-Vp	3	396 ± 72	76
5	-Dlv	3	429 ± 74	82
6	-Dm	4	394 ± 63	75

TABLE 1—*Continued*

No.[a]	Area[b]	N^c	Amount of catecholestrogen[d]	Prop. distr.[e]
7	-Dd	3	395 ± 30	75
8	-Dd + Dld	4	380 ± 70	72
9	Met-C	6	193 ± 36	37
8– 1	Di-NPO	6	397 ± 76	76
2	-NAH + Tr Olf	6	233 ± 45	44
3	Tel-Vp	3	411 ± 38	78
4	-Dlv	5	358 ± 64	68
5	-Dm	4	319 ± 21	61
6	-Dd	3	305 ± 55	58
7	-Dd + Dlv	3	376 ± 34	72
9– 1	Di-NPO + NAP	5	395 ± 80	75
2	-NDTL	3	275 ± 53	52
3	-NAH	3	327 ± 16	62
4	-NPO	5	396 ± 62	75
5	-Tr O	3	254 ± 18	48
6	Tel-Dm	5	482 ± 70	92
7	-Dm	5	401 ± 32	76
8	-Dlv	4	413 ± 34	79
10– 1	Di-NLT	6	415 ± 75	79
2	-NAT	4	354 ± 59	67
3	-NDLI + NAH	3	348 ± 38	66
4	-NAP + NVM	5	318 ± 66	61
5	Mes-TO	5	356 ± 69	68
6	Di-NH	3	415 ± 56	79
7	Mes-TO	3	451 ± 60	86
8	-TO	5	516 ± 64	98
9	Met-C	5	226 ± 40	43
11– 1	Di-NLT	6	407 ± 74	78
2	-NAT	3	253 ± 44	48
3	-NDLI	3	355 ± 45	68
4	-NPoP + NVM	5	307 ± 49	59
5	-NDL + AP	5	239 ± 41	46
6	Mes-Ts + TO	3	346 ± 54	66
7	-TO	5	368 ± 67	70
8	Di-NH + NDM	4	307 ± 57	59
9	Mes-TO	3	259 ± 14	49
10	-TO	6	389 ± 46	74
12– 1	Di-NLT + NRL	6	310 ± 30	59
2	-NAT	4	245 ± 40	47
3	-NDLI	3	375 ± 21	71
4	-NPoP + NVM	5	391 ± 58	75
5	-NPG	4	226 ± 37	43
6	Mes-TS	4	370 ± 65	70
7	-TS + TO	3	392 ± 29	75
8	Di-NDM	6	278 ± 50	53
9	Mes-TO	3	378 ± 2	72
10	-TO	5	433 ± 46	83
13– 1	Di-NLT + NRL + NAT	6	355 ± 42	68
2	-NRL + NAT	5	279 ± 46	53
3	-NDLI	5	278 ± 51	53
4	-NPoP	5	448 ± 62	85
5	-NPG	4	253 ± 50	48
6	Mes-TM	5	292 ± 33	56
7	-TS	6	277 ± 53	53

TABLE 1—*Continued*

No.[a]	Area[b]	N[c]	Amount of catecholestrogen[d]	Prop. distr.[e]
9	-TO + TS	5	403 ± 35	77
14– 1	Di-NLT + NRP	4	377 ± 63	72
2	-NRL	4	298 ± 47	57
3	-NDLI	4	293 ± 51	56
4	Mes-TM	3	272 ± 22	52
5	-NG + TM	4	187 ± 33	36
6	-TS	5	306 ± 45	58
7	-TS	6	441 ± 45	84
8	-TM	5	209 ± 40	40
9	Met-C	5	271 ± 50	52
15– 1	Di-NDLI	5	278 ± 64	53
2	-NDLI + NRL	5	293 ± 50	56
3	-NDLI	4	253 ± 36	48
4	-NRP	6	472 ± 58	90
5	Mes-TM	5	262 ± 24	50
6	-TM	6	193 ± 46	37
7	-TS	6	263 ± 49	50
8	-TS	5	454 ± 99	87
16– 1	Di-NDLI	3	369 ± 98	70
2	-NDLI	5	235 ± 55	45
3	-NDLI	5	336 ± 57	64
4	Di-CM	4	238 ± 32	45
5	Mes-TM	5	181 ± 38	35
6	-TS	3	276 ± 52	53
7	-TS	3	333 ± 27	64
17– 1	Di-NDLI	6	302 ± 59	58
2	-NDLI	3	192 ± 27	37
3	Mes-TM	6	139 ± 24	27
4	-TM	5	201 ± 52	38
5	-TS	5	391 ± 44	75
18– 1	Met-	4	118 ± 26	23
2	Met-	3	161 ± 71	30
3	Met-C	5	281 ± 62	53

[a] Numbers corresponding to the transverse sections and their punches, as given in Fig. 3.

[b] Neuroanatomical areas. Abbreviations used: Tel, telencephalon; Di, diencephalon; Mes, mesencephalon; Met, metencephalon; AP, area pretectalis; C, cerebellum; CA, commissura anterior; CH, commissura horizontalis; CM, corpus mamillare; CP, commissura posterior; Dc, area dorsalis telencephali pars centralis; Dd, area dorsalis telencephali pars dorsalis; Dl, area dorsalis telencephali pars lateralis; Dld, area dorsalis telencephali pars lateralis dorsalis; Dlv, area dorsalis telencephali pars lateralis ventralis; Dm, area dorsalis telencephali pars medialis; Dp, area dorsalis telencephali pars posterior; H, hypophysis; nV, nervus trigeminus; NAH, nucleus anterior hypothalami; NAP, nucleus anterior periventricularis; NAT, nucleus anterior tuberis; NDL, nucleus dorsolateralis thalami; NDLI, nucleus diffusus lobi inferioris; NDM, nucleus dorsomedialis thalami; NDTL, nucleus diffusus tori lateralis; NG, nucleus glomerulosus; NH, nucleus habenularis; NLT, nucleus lateralis tuberis; NLV, nucleus lateralis valvulae; NMnV, nucleus motorius nervi trigemini; NPG, nucleus preglomerulosus; NPO, nucleus preopticus; NPoP, nucleus posterior periventricularis; NRL, nucleus recessus lateralis; NRP, nucleus recessus posterioris; NT, nucleus tenia; NVM, nucleus ventromedialis thalami; TM, tegmentum mesencephali; TO, tectum opticum; TS, torus semicircularis; Tr O, tractus opticus; Tr Olf, tractus olfactorius; Vd, area ventralis telencephali pars dorsalis; Vl, area ventralis telencephali pars lateralis; Vp, area ventralis telencephali pars postcommissuralis; Vv, area ventralis telencephali pars ventralis; Vs, area ventralis telencephali pars supracommissuralis.

[c] Number of determinations.

[d] Amount of catecholestrogen in femtomole · milligram^{-1} · hour^{-1} (means ± SEM).

[e] Proportional distribution of the formed catecholestrogen related to the ventrodorsal area of the telencephalon (section 5, punch 4: 100%).

17). A moderate and low activity was measured in the tegmentum (TM) (sections 13–17).

The estrogen-2-hydroxylase activity in punches of the metencephalic cerebellum (Met-C) (sections 4, 7, 10, 14, 18) varied between low and moderate; and in the hindbrain (Met-) (section 18) the lowest activity was measured.

DISCUSSION

The combination of microanatomical and biochemical methods made it possible to localize fairly precisely the active form of the enzyme estrogen-2-hydroxylase in the brain of the African catfish, C. gariepinus. Although this enzyme could be demonstrated throughout the brain, some parts of the fore- and midbrain showed a higher enzyme activity than others. In the telencephalon a high enzyme activity was found in the rostral and caudal part. In the diencephalon the entire magnocellular part of the preoptic nucleus, the rostral part of the nucleus anterior periventricularis, the nucleus lateralis tuberis, the rostral part of the nucleus habenularis and of the nucleus anterior tuberis, the caudal part of the nucleus posterior periventricularis, and the nucleus recessus posterioris showed a high enzyme activity. In the mesencephalon a high activity was detected in the tectum opticum and the dorsolateral part of the torus semicircularis.

In a previous study the activity of the enzyme aromatase, catalyzing the conversion of androgens into estrogens, was also localized in the brain of the male African catfish (Timmers et al., 1987). Comparing the distribution of both enzymes it can be concluded that a high aromatase activity is associated with most of the areas with a more than moderate 2-hydroxylase activity. Exceptions are brain areas containing the nuclei NH, NAT, NRL, and NRP, where a low to moderate aromatase activity prevailed.

The significance of 2-hydroxylase activity in regions without or with a hardly detectable aromatase activity, particularly the cerebellum, is not clear. Indeed the appropriate substrates, namely estradiol and estrone, are not formed by the testes and seminal vesicles (Schoonen and Lambert, 1986a, b) and are therefore not available for these parts of the brain; the more so since transportation of estrogens from areas where aromatase activity has been demonstrated is not very likely. Therefore, the possibility exists that this enzyme catalyzes the catechol formation of substrates different from estrogens, e.g., tyrosine.

Some brain regions with both a high aromatase and a high 2-hydroxylase activity, i.e., the telencephalon, the preoptic area, and the area tuberalis, are known to be involved in reproductive processes, as deduced from (1) lesion experiments (Peter and Crim, 1978; Kyle and Peter, 1982; Satou et al., 1982; Koyama et al., 1984); (2) effects of electrical stimuli (Demski and Knigge, 1971; Demski et al., 1975; Satou et al., 1982, 1984; Koyama et al., 1985); (3) the presence of LHRH-immunoreactive material (Goos and Murathanoglu, 1977; Pan et al., 1979; Schreibman et al., 1979; Borg et al., 1982; Kah et al., 1982, 1984; Halpern-Sebold and Schreibman, 1983; Demski, 1984; Goos et al., 1985a; Nozaki et al., 1985; Nunez-Rodriguez et al., 1985); and (4) the presence of androgen-concentrating neurons (Davis et al., 1977; Demski, 1978, 1984).

Thus it seems that certain regions involved in reproductive processes possess a high aromatase and a high 2-hydroxylase activity. Inversely, it may be postulated that if a high enzyme activity is present in certain brain areas, an involvement in reproductive processes can be expected. So, the tectum opticum and the torus semicircularis of the African catfish might be involved in reproduction. The tectum opticum of teleosts is an important center for receiving and processing visual information

(Northcutt, 1983), and the torus semicircularis is a center for neural processing of auditory, lateral line, and electrical stimuli (Northcuttt, 1981; Schellart and de Wolf, 1982; Knudsen, 1977, 1978). An involvement in reproduction of these regions can be deduced from studies of the toadfish, *Opsanus tau* (Fine *et al.*, 1982), which showed that these mesencephalic centers can bind androgens; from studies of other species, which showed that they contain LHRH immunoreactive fibers, especially in the tectum opticum (Münz *et al.*, 1981; Kah *et al.*, 1982, 1984; Nozaki *et al.*, 1985; Nunez-Rodriguez *et al.*, 1985); and also from the neuroethological studies of Satou (1987), who suggested that tectum opticum and torus semicircularis may be candidates for centers for male spawning behavior in the chum salmon, *Oncorhynchus keta*.

The coexistence of the enzymes aromatase and 2-hydroxylase in several brain regions of the male African catfish makes it possible that androgens can be metabolized into catecholestrogens. Catecholestrogens can have several biological effects (for review see Ball and Knuppen, 1980). In mammals they can inhibit prolactin secretion (Barbieri *et al.*, 1980), induce sexual behavior and brain differentiation (Jellink *et al.*, 1981), interact with estrogen receptors (Merriam *et al.*, 1980), and also influence gonadotropin (GTH) secretion (Parvizi and Ellendorff, 1980; MacLusky *et al.*, 1981).

The influence on the GTH secretion, however, has a dual character. Naftolin *et al.* (1975a) observed a rise in serum luteinizing hormone (LH) in immature male rats after treatment with catecholestrone, whereas Okatani and Fishman (1984) could demonstrate an inhibiting effect of catecholestrone on the LH surge in adult 4-day cycling rats. A decrease in LH secretion was also demonstrated by Parvizi and Ellendorff (1975) in castrated adult pigs 2 hr after an injection with catecholestradiol directly into the amygdala.

In teleosts, however, no data are available about the effects of catecholestrogens on GTH release, but the catecholestrogen synthesis in brain regions involved in the regulation of GTH secretion in African catfish supports our hypothesis that catecholestrogens play an intermediate role in the negative feedback mechanism of estrogens and aromatizable androgens by an indirect influence on the methylation (inactivation) of dopamine (Fig. 1).

The methylation of catecholestrogens on the one hand and of dopamine on the other, mediated by COMT, may be an essential step in the biochemical link between sex steroids and dopamine. For this to occur it is necessary that the enzyme COMT be present in specific brain areas. Second, the enzyme must be capable of methylating both catechol substrates, i.e., catecholestrogen and dopamine. Third, this methylation should be competitive. Studies have been carried out regarding the presence and the kinetic behavior of the enzyme COMT in the brain of male African catfish (Timmers and Lambert, 1988). It can be concluded from these studies that COMT present in the catfish brain not only enables the conversion of catecholestrogens into methoxyestrogens, but also can methylate dopamine into methoxytyramine. The competitive behavior concerning the methylation of both catechol substrates will be the subject of further studies.

ACKNOWLEDGMENTS

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