is considerably more adultlike than was previously reported. We conclude that both the partial purification of estrogen receptor and the elimination of AFP before labeling makes more direct assessment of hormone affinity possible. In addition to the quantitative similarities, our data indicate that the estradiol-binding macromolecules from embryonic hypothalamus and "brain" are also qualitatively adultlike. Embryonic and prepubertal estrogen receptors adhere to DNA-cellulose, exhibit characteristic patterns when eluted from DNA-cellulose with a linear concentration gradient of NaCl, and are sensitive to DES.

In summary, we believe these data represent the most comprehensive analysis to date of embryonic estrogen receptors from brain of any mammalian species. This analysis was made possible by a new application of DNA-cellulose affinity chromatography which can be readily adapted (*17*) to analysis of estrogen receptors from other biological systems in which removal of nonreceptor estrogen-binding macromolecules is essential.

The masculinizing effects of prenatal exposure to sex hormones have been well documented in rodents (*1, 2*). The criticism can be raised, however, that sex hormones might be retained by embryos and act subsequently in the postnatal brain to produce these effects. However, our demonstration of putative estrogen receptors in embryonic mouse brain suggests that sex hormones might act prenatally, since estrogen receptors might mediate the actions of both estrogens and androgen metabolites (*18*). Together with our observations that embryonic mouse brain also contains putative androgen receptor (*5*) and that embryonic rat brain contains estrogen receptor (*19*), it now appears possible that embryonic rodent brain has the biochemical potential to respond to its sex steroid environment.

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References and Notes

1. C. A. Barraclough and J. H. Leathem, *Proc. Soc. Exp. Biol. Med.* **85**, 673 (1954); A. A. Gerall and I. L. Ward, *J. Comp. Physiol. Psychol.* **62**, 370 (1966); C. A. Barraclough, *Neuroendocrinology* (Academic Press, New York, 1967), pp. 62–99.
2. R. Gandelman, F. S. vomSaal, J. M. Reinisch, *Nature (London)* **266**, 722 (1977); L. G. Clemens, B. A. Gladue, L. P. Coniglio, *Horm. Behav.* **10**, 40 (1978).
3. T. O. Fox, *Nature (London)* **258**, 441 (1975).
4. ———, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4303 (1975).
5. ———, C. C. Vito, S. J. Wieland, *Am. Zool.* **18**, 525 (1978).
6. B. Attardi and S. Ohno, *Endocrinology* **99**, 1279 (1976).
7. J. Barley, M. Ginsburg, B. D. Greenstein, N. J. MacLusky, P. J. Thomas, *Nature (London)* **252**, 259 (1974); B. R. Westley *et al.*, *Brain Res.* **113**, 441 (1976).
8. B. S. McEwen, L. Plapinger, C. Chaptal, J. Gerlach, G. Wallach, *Brain Res.* **96**, 400 (1975); J. Kato, *Ann. Biol. Anim. Biochim. Biophys.* **16**, 467 (1976).
9. For hypothalamic extracts, estrogen receptor adheres to DNA-cellulose and elutes as the 4*S* cytosol form, unless heat is applied [T. O. Fox, *Brain Res.* **120**, 580 (1977)]. Since the free 4*S* estrogen receptor of uterus adheres to DNA at low salt concentration (150 m*M* NaCl) nearly as well as the hormone-complexed form [K. R. Yamamoto and B. M. Alberts, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2105 (1972)], we expected the unbound hypothalamic receptor also to adhere to DNA-cellulose. This is unlike the formation of 5*S* uterine receptor, which they show to be estrogen-dependent. Indeed, at low ionic strength (50 m*M* NaCl), prepubertal receptor from hypothalamus adheres to DNA-cellulose equally well with and without bound ligand. Similar proteins in embryonic brain tissues should adhere to DNA-cellulose under these conditions and elute with the same properties.
10. T. O. Fox, in *Proceedings of the Second Annual (1976) Maine Biomedical Science Symposium*, S. R. Zeli, Ed. (Univ. of Maine Press, Orono, 1977), pp. 544–572.
11. L. Plapinger and B. S. McEwen, *Steroids* **26**, 255 (1975); B. Attardi and E. Ruoslahti, *Nature (London)* **263**, 685 (1976); R. H. Benno and T. H. Williams, *Brain Res.* **142**, 182 (1978).
12. T. O. Fox and C. Johnston, *Brain Res.* **77**, 330 (1974).
13. C. C. Vito and T. O. Fox, unpublished data.
14. N. J. MacLusky and B. S. McEwen, *Nature (London)* **274**, 276 (1978).
15. Saturating concentrations of [^{3}H]estradiol are defined as those concentrations at which macromolecular binding is ≳ 99 percent of the absolute maximum. Under the experimental conditions described for Fig. 1, maximum binding is essentially complete within 1 hour at the estradiol concentrations indicated for both embryonic and adult brain extracts.
16. J. A. Katzenellenbogen *et al.*, *Biochemistry* **14**, 2310 (1975).
17. We have demonstrated that an estrogen receptor like that in mammals is present in cytosol extracts of turtle oviduct (A. Salhanick, C. Vito, T. Fox, I. Callard, *Endocrinology*, in press). Furthermore, we have analyzed the estradiol-binding affinity as well as the steroid-binding specificity of this estrogen receptor. Previous attempts to analyze this receptor were impaired by the presence of a high-capacity, steroid-binding plasma protein in cytosol extracts. Because nonreceptor estradiol-binding proteins from turtle oviduct, as well as those from several other species (*10*), do not adhere to DNA-cellulose under our experimental conditions, it is possible to selectively assay estrogen receptor from preparations containing both receptor and nonreceptor estradiol-binding proteins. Even in the case of fetal guinea pig brain, which lacks a high-capacity estrogen-binding protein such as AFP, the existence of "receptor-like" macromolecules [L. Plapinger, I. T. Landau, B. S. McEwen, H. H. Feder, *Biol. Reprod.* **16**, 586 (1977)] is not agreed upon [J. R. Pasqualini, C. Sumida, B. L. Nguyen, C. Gelly, *J. Steroid Biochem.* **9**, 443 (1978)]. Other estrogen-binding components complicate the interpretation of their respective quantitative binding and sedimentation data. These problems might be resolved by a qualitative elution analysis of presumed receptors from DNA-cellulose.
18. B. R. Westley and D. F. Salaman, *Brain Res.* **119**, 375 (1977).
19. C. C. Vito and T. O. Fox, *Neurosci. Abstr.* **3**, 359 (1977).
20. We thank S. J. Wieland for his advice and encouragement. Portions of this work were supported by a grant from The National Foundation–March of Dimes, a NIH postdoctoral training grant NINCDS-NS-07017, and an NICHD postdoctoral fellowship to C.C.V., and by NIH grant HD 10818 and a research career development award to T.O.F.

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Teleostean Urophysis: Urotensin II and Ion Transport Across the Isolated Skin of a Marine Teleost

Abstract. *The caudal neurosecretory peptide urotensin II rapidly inhibits by 30 percent the short-circuit current across the isolated skin of a marine teleost. The effect appears to be specific and cannot be attributed to actions of epinephrine, urotensin I, or arginine vasotocin. The results strongly suggest that urotensin II may act directly on ion-transporting cells involved in teleostean hypoosmoregulation.*

The caudal neurosecretory system, including its neurohemal organ, the urophysis, is unique to fishes and is now known to produce at least two biologically active peptides, designated urotensins I and II (UI and UII), as well as specific carrier proteins, the urophysins (*1*). Ten years ago, two effects of the urophysis were reported: stimulation of water absorption by the toad bladder (*2*) and stimulation of contraction of the trout urinary bladder (*3*). Both of these effects had possible implications for a role of the urophysis in osmoregulation. The former effect is now considered to be due to a factor indistinguishable from arginine vasotocin (AVT) found only in some teleostean urophyses (*1*) and the latter to the general spasmogenic action of UII in fishes (*1*). A relationship between the urophysis and osmoregulation in teleosts has long been suggested (*4*); however, no direct effect of urophysial peptides on transport processes has yet been shown. Here we report an unequivocal effect of UII on ion transport across the isolated skin of the long-jawed goby *Gillichthys mirabilis*, a euryhaline marine teleost.

We have observed that *Gillichthys* skin actively excretes chloride ions, when bathed on both sides with Ringer solution in an Ussing-style membrane chamber with the spontaneous transepithelial potential difference (*PD*) clamped to zero (*5*). Further, the short-circuit current (I_{sc}) at zero *PD* was found to equal the net efflux of chloride, as monitored by radioisotope fluxes, whereas there is no net flux of sodium

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Table 1. Effect of urotensin II from *Gillichthys* and *Catostomus* on *PD*, I_{sc}, and *R* of *Gillichthys* skin, expressed as the mean percentage change relative to control skins plus or minus the standard error. The number of experiments is given in parentheses.

Urotensin II	*PD* (%)	I_{sc} (%)	*R* (%)
Gillichthys UII			
1 mU/ml		−22 ± 4 (8)	
10 mU/ml	−26 ± 5 (15)	−32 ± 3 (16)	12 ± 4 (15)
Catostomus UII			
1 mU/ml	−9 ± 3 (6)	−8.5	0 ± 4 (6)
10 mU/ml	−24 ± 4 (11)	−31 ± 4 (12)	32 ± 6 (10)

across the tissue under these conditions (*6*).

The skin, containing numerous mitochondria-rich cells (*6*) similar to the chloride cells found in the gill epithelium of marine teleosts, is thus a model for extrarenal salt excretion in fishes. Not only is the flat configuration of the isolated skin an advantage over the highly convoluted gill epithelium, but also the skin model allows one to examine the direct effects of hormones without complications due to the vasoactivity of these substances, since the epithelium is dissected free of underlying blood vessels prior to mounting in vitro. For instance, we have shown that epinephrine, a potent vasodilator of the gill vasculature (*7*), rapidly reduces the *PD*, I_{sc}, and net efflux of chloride across the skin via stimulation of α-aminergic receptors (*6*). Similar findings have been reported with *Fundulus* subopercular epithelium, another flat membrane containing chloride cells (*8*). Inasmuch as the pituitary appears to maintain skin ion transport over long periods (*6*), both long-term and short-term hormonal controls over the skin's osmoregulatory function are indicated.

Urotensin II, purified from the urophyses of both *Gillichthys* and the white sucker, *Catostomus commersoni* (a stenohaline freshwater teleost), inhibits the *PD* and I_{sc} across *Gillichthys* skin, compared with control membranes (Table 1; Fig. 1). The reduction in *PD* and I_{sc} was accompanied by a moderate increase in the transepithelial resistance (*R*). The UII effect commenced within 2 to 6 minutes after addition of the hormone to the serosal bathing solution, similar to the 2- to 3-minute delay previously observed for the onset of epinephrine inhibition (*6*). Steady-state I_{sc} was reached 10 to 30 minutes after addition of UII. The threshold dose, approximately 1 mU/ml, is much higher than that in the smooth muscle–contracting bioassay for UII [0.1 mU/ml (*9*)]. However, the dose-response curve for the skin (Fig. 1), like that in the bioassay, is steep, and maximal doses are 10 to 20 times the threshold dose.

In view of the presence of AVT-like hydroosmotic activity in *Gillichthys* urophyses (*10*), two skins were exposed to 500 n*M* AVT (0.5 μg/ml) with resultant decreases in I_{sc} of 8 and 14 percent. However, the inhibitory effect of the two UII preparations cannot be attributed to AVT contamination since *Gillichthys* UII at 10 mU/ml would contain an estimated 5 to 7 p*M* AVT (*9*) and *Catostomus* urophyses appear to lack AVT altogether (*1*). Purified *Catostomus* UI at 10 mU/ml had little effect on the *PD* of four skin pairs (percentage change compared to controls, +7 ± 2.7). Thus the described inhibition of the skin ion transport appears to be specific for UII.

Although the UII effect is only partially reversed by rinsing of the tissue with Ringer solution, the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (IBMX) at 2×10^{-5} or 5×10^{-5}*M* caused a larger absolute and percentage increase in the *PD* and I_{sc} in UII-inhibited skins (Fig. 1) (*11*). The reversal by IBMX of the UII-initiated drop in I_{sc} implies that the UII effect is not mediated by increased intracellular adenosine 3′,5′-monophosphate (cyclic AMP) since IBMX itself has this effect (*12*). On the contrary, we believe that UII may act through another intracellular messenger, possibly guanosine 3′,5′-monophosphate (cyclic GMP). The enhancement of *PD* and I_{sc} by IBMX in the control skins (Fig. 1) suggests that cyclic AMP may be important in "turning on" the transepithelial ion transport.

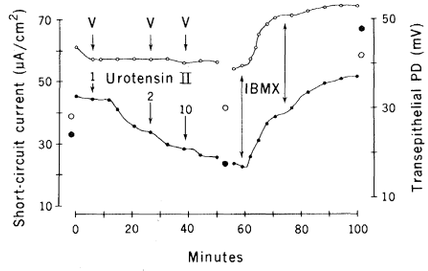

Fig. 1. Cumulative addition of purified *Gillichthys* UII to the serosal bathing solution of the isolated *Gillichthys* skin inhibits the *PD* (●) and I_{sc} (•—•) compared with the *PD* (○) and I_{sc} (○—○) of the paired control skin, which received only the hormone vehicle (*V*). The phosphodiesterase inhibitor IBMX was added in two steps (2×10^{-5} and 3×10^{-5}*M*) and caused greater increases in *PD* and I_{sc} in the experimental skin.

Our data demonstrate UII activity on a teleost ion-transporting epithelium, but the physiological importance of this finding requires consideration. In view of the secretion of the urophysial neurohormones into the caudal vein–renal portal system (*13*), we do not know the quantity of UII available to the skin through the systemic circulation. On the other hand, osmoregulatory organs more directly supplied by blood draining the urophysis—the kidney and possibly the urinary bladder—could be influenced by physiological concentrations of urophysial hormones.

Note added in proof: Richman and Barnawell and Edwards and Larsen (*14*) recently reported (in abstract form) sodium transport effects of crude urophysial extracts on the catfish intestine and frog skin, respectively. These observations further support the notion of ion transport regulation by urotensins, although the possible presence of AVT in their urophysial extracts should be considered [see (*10*)].

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References and Notes

1. H. A. Bern and K. Lederis, in *Neurosecretion and Neuroendocrine Activity: Evolution, Structure and Function*, W. Bargmann, A. Oksche, A. Polenov, B. Scharrer, Eds. (Springer-Verlag, New York, 1978), pp. 341–349; D. K. O. Chan and H. A. Bern, *Cell Tissue Res.* **174**, 339 (1976); K. Lederis, *Am. Zool.* **17**, 823 (1977).
2. F. Lacanilao, *Science* **163**, 1326 (1969).
3. K. Lederis, *ibid.*, p. 1327. Bladder contractility can affect hydromineral balance by altering the time in which urinary components can be reabsorbed by the bladder [see T. Hirano, D. W. Johnson, H. A. Bern, S. Utida, *Comp. Biochem. Physiol. A* **45**, 529 (1973)].
4. H. A. Bern, *Science* **158**, 455 (1967); ____ and R. S. Nishioka, *Gunma Symp. Endocrinol.*, in press. Recent evidence for this relationship can be found in G. Chevalier, *Gen. Comp. Endocrinol.* **29**, 441 (1976); J. N. Fryer, N. Y. S. Woo, R. L. Gunther, H. A. Bern, *ibid.* **35**, 238 (1978); T. Hirano, *Gunma Symp. Endocrinol.*, in press.
5. W. S. Marshall, *J. Comp. Physiol.* **114**, 157 (1977). We are now using paired pieces of skin that are located on the sides of the head and extend to the tips of the long premaxillary bones of *Gillichthys*. Although ion transport is present across the buccal epithelium and the skin of the lower jaw, the described area is the largest and most convenient area of scaleless skin on the animal.
6. W. S. Marshall and H. A. Bern, in *Epithelial Transport in the Lower Vertebrates*, B. Lahlou, Ed. (Cambridge Univ. Press, New York, in press).
7. C. M. Wood and G. Shelton, *J. Exp. Biol.* **63**, 505 (1975); P. Payan and J.-P. Girard, *Am. J. Physiol.* **232**, H18 (1977); S. Dunel and P. Laurent, *C.R. Acad. Sci. Ser. D* **284**, 2011 (1977).

8. K. J. Degnan, K. J. Karnaky, Jr., J. A. Zadunaisky, *J. Physiol. (London)* **271**, 155 (1977); K. J. Karnaky, Jr., K. J. Degnan, J. A. Zadunaisky, *Science* **195**, 203 (1977).
9. D. K. O. Chan, R. Gunther, H. A. Bern, *Gen. Comp. Endocrinol.* **34**, 347 (1978). In this assay, first developed by P. R. Zelnik and K. Lederis [*ibid.* **20**, 392 (1973)], trout posterior intestine is used.
10. F. Lacanilao, *ibid.* **19**, 405 (1972); *ibid.*, p. 413.
11. We also observed reversal of epinephrine inhibition of the I_{sc} by 5×10^{-5}*M* IBMX.
12. J. A. Beavo, N. L. Rogers, O. B. Crofford, J. G. Hardman, E. W. Sutherland, E. V. Newman, *Mol. Pharmacol.* **6**, 597 (1970).
13. G. Fridberg and H. A. Bern, *Biol. Rev.* **43**, 175 (1968).
14. N. H. Richman and E. B. Barnawell, *Am. Zool.* **18**, 651 (1978); S. C. Edwards and J. B. Larsen, *ibid.*, p. 577.
15. We are grateful to M. Barkley and the late I. I. Geschwind for *Gillichthys* UII and to K. Lederis for *Catostomus* UI and UII. We also thank T. Machen and R. Gunther for advice during the experiments. Supported by National Research Council of Canada and Killam (Canada) postdoctoral fellowships to W.S.M. and by NSF grant PCM-16345 to H.A.B.

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Multiple Representations of the Body Within the Primary Somatosensory Cortex of Primates

Abstract. *Microelectrode mapping experiments indicate that the classical primary somatosensory cortex of monkeys consists of as many as four separate body representations rather than just one. Two complete body surface representations occupy cortical fields 3b and 1. In addition, area 2 contains an orderly representation of predominantly "deep" body tissues. Area 3a may constitute a fourth representation.*

Many of our current concepts of the organization of the somatosensory cortex in primates stem from the early studies of C. N. Woolsey and his colleagues, first published more than 40 years ago (*1*).

Using surface-evoked potential methods, these pioneering investigators concluded that there was a large single representation of the contralateral body surface within the anterior parietal cortex of macaque monkeys. The "representation" [later termed SI (*2*)] included four distinct cytoarchitectonic fields, areas 3a, 3b, 1, and 2 of Brodmann (*3*). The basic organization of the representation was later summarized by Woolsey for several primate species by a "homunculus," a distorted drawing of the body surface reflecting the proportions of different skin surfaces in SI as well as the overall somatotopic organization of SI (*4*). This body figure was drawn with the digits of the hand and foot represented rostrally in the parietal cortex, the back caudally, the tail medially, and the face laterally (Fig. 1A).

Observations recorded in later single unit studies (*5*) were not consistent with the concept of SI as a simple, continuous somatotopic representation of the contralateral body surface. Cutaneous receptors signaling light touch were reported to provide the major input to area 3b; mixed cutaneous and deep receptor input activated mosaically distributed neuron groups in area 1; and deep receptor input was predominant in area 2. Thus, if there was a single body surface representation in SI (as portrayed by a homunculus overlying the architectonic fields), different regions of the body surface would relate to quite different classes of neurons in SI.

Powell and Mountcastle (*5*) suggested a second concept of SI that appeared to be more compatible with the uneven cortical distribution of receptor inputs than the homunculus concept. They noted that the same body region could activate neurons in different electrode penetrations across the rostrocaudal dimension of SI. Thus, a reasonable alternative to the homunculus concept was that any given body region be represented within a rostrocaudal band extending across areas 3a, 3b, 1, and 2. All body surface locations would thereby be subserved by peripheral receptors of all classes, and areas 3a, 3b, 1, and 2 would be considered parts of a single representation. Variants or aspects of the rostrocaudal bands concept have been forwarded in more recent investigations of SI organization in spider, squirrel, and macaque monkeys (*6*) (Fig. 1B).

A third view of SI organization was suggested by the microelectrode mapping studies of Paul, Merzenich, and Goodman (*7*) who described two "complete" representations of the glabrous hand within SI of macaque monkeys; one representation was within area 3b, the other was related to area 1. Area 3a was not included in either representation, and there was partial evidence for a third representation in area 2. Although the organizations of areas 3b, 1, and 2 were not further investigated, these stud-

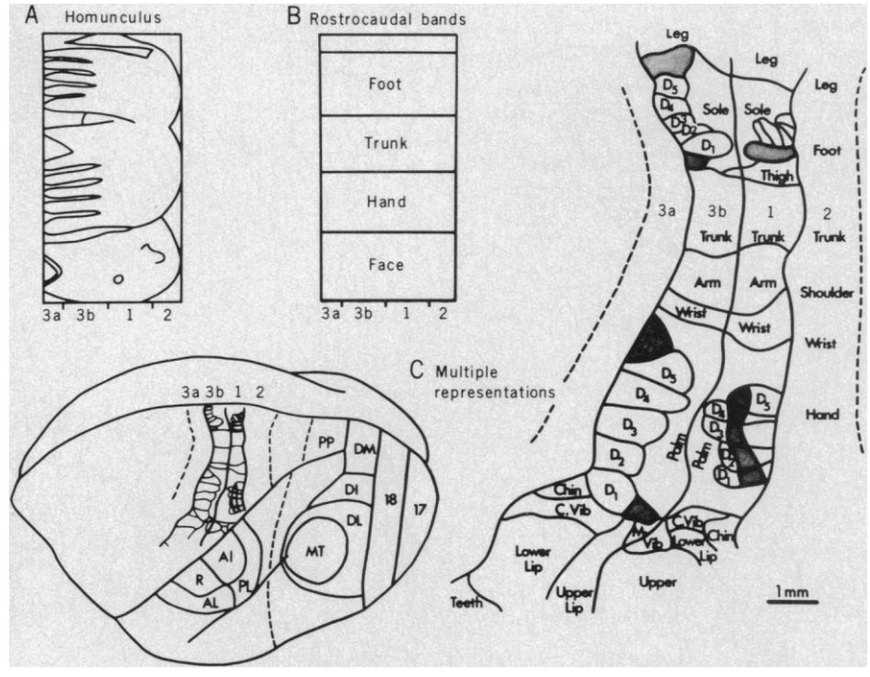

Fig. 1. Three conceptions of the organization of postcentral parietal somatosensory cortex (SI). (A) A distorted body figure (homunculus) over the four architectonic fields. In this view a single topographic body representation constitutes SI. (B) The SI as rostrocaudal bands. Major body parts are represented in all architectonic fields. (C) Multiple representations of the body within the cortex formerly designated as SI. Each architectonic field contains a representation. The organizations of the two cutaneous representations, SI proper (3b) and the posterior cutaneous field (1), and some of the organizations of the area 2 representation are shown for the owl monkey (*Aotus*). Sectors within each map limit the representations of body parts. The digits of the foot (upper) and hand (lower) are numbered, and the dorsal hairy surfaces are shaded. Chin and mandibular vibrissae are indicated. The positions of the fields on the brain are shown on the lower left. Visual and auditory areas are also shown (*14*).

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