ed by injecting a mouse with the human enzyme.

Our identification of a monoclonal antibody that discriminates between MAO-A and -B is consistent with recent evidence of structural differences in the active site subunits of the two species (*8, 9, 20*). Our results also agree with McCauley and Racker's (*11*) finding that bovine liver MAO-B has at least one antigenic site not found on bovine liver MAO-A. Conversely, Powell and Craig (*12*) have prepared antiserums that react preferentially with human MAO-A. Our results do not support the conclusion of Dennick and Mayer (*13*) or Russell *et al.* (*21*) that human liver MAO-A and -B are immunologically indistinguishable. It should be noted, however, that these investigators raised antiserums against MAO purified from liver mitochondria, which contain both MAO-A and -B. It is possible that their purified MAO contained a mixture of inactive MAO-A and active MAO-B (*22*), and therefore elicited antiserums containing a mixture of antibodies to MAO-A and antibodies to MAO-B. Such antiserums could inhibit both MAO-A and -B even if no single antibody recognized both molecules. In view of these uncertainties, additional monoclonal antibodies should be evaluated for their ability to discriminate MAO-A and -B.

Correlations between the level of MAO-B activity in human platelets and a variety of psychiatric and neurological disorders have been reported by numerous investigators (*23*), but there has been no method for determining whether the low MAO-B activities observed in some individuals are due to low amounts of normal enzyme or normal amounts of altered enzyme. Preliminary experiments show that crude extracts of human liver and platelets inhibit the binding of [^{3}H]pargyline-labeled MAO-B to MAO-1C2 and suggest that this competition can form the basis for a highly sensitive quantitative assay for MAO-B protein in these extracts.

The ready availability and high specificity of antibody MAO-1C2 should prove valuable in a wide range of studies of human MAO-B, including measurement of MAO protein in platelets from patients with neurological and psychiatric disorders and preparation of human MAO-B suitable for structural studies.

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Brain Target Sites for 1,25-Dihydroxyvitamin D_3

Abstract. *Autoradiographic studies with ^{3}H-labeled 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] demonstrate, in certain neurons of rat forebrain, hindbrain, and spinal cord, a nuclear retention and concentration of radioactivity, which can be prevented by treatment with 1,25(OH)$_2$D$_3$, but not with 25-hydroxyvitamin D$_3$. These results indicate the presence of brain receptors in addition to pituitary receptors for 1,25(OH)$_2$D$_3$ and suggest a central modulation of calcium homeostasis and other central effects for this hormone. The existence of a brain-pituitary axis for certain 1,25(OH)$_2$D$_3$-mediated endocrine-autonomic effects is postulated.*

Calcium homeostasis was generally not considered to be centrally regulated until 1979, when evidence for a direct central regulation was provided by our autoradiographic studies. After radioactively labeled 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] was injected in rats fed a vitamin D–deficient diet, radioactivity was found to be concentrated in the nuclei of certain cells in the pituitary pars distalis and infundibular process (*1*). Target cells in the anterior pituitary were identified, through combined autoradiography and immunohistochemistry, as thyrotrophs (*2*). Subsequently, biochemical studies demonstrated binding proteins for 1,25(OH)$_2$D$_3$ in a pituitary tumor cell line and in homogenates of whole pituitaries, without consideration of the specific pituitary lobes or cell types (*3*). The biochemical studies did not provide evidence of receptors for 1,25(OH)$_2$D$_3$ in rat brain (*3, 4*); in contrast, our autoradiographic experiments revealed neuronal target sites for this steroid hormone in the brain and spinal cord (*5*), as well as 25 new target cell types in peripheral organs (*6*).

Nine 21-day-old male Holtzman Sprague-Dawley rats were fed a vitamin D–deficient diet (*7*) for 5 to 6 weeks and kept at a photoperiodic cycle of 12 hours of light and 12 hours of darkness. All of the animals were given intravenous injections (0.19 μg per 100 g of body weight) of 1,25(OH)$_2$[26,27-^{3}H]D$_3$ (specific activity 160 Ci/mmole), dissolved in 75 percent ethanol–isotonic saline. Three

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of these animals were injected intravenously with 10 μg of unlabeled $1,25(OH)_2D_3$ and one was injected with 10 μg of 25-hydroxyvitamin D_3 (25-OH-D_3) 30 minutes before injection of the labeled compound. Two hours after injection of the labeled compound, the animals were killed. Brain, cervical spinal cord, pituitary, and intestine were dissected. Samples were mounted and frozen onto tissue holders, sectioned in a cryostat at 4 μm, and thaw-mounted on slides coated with photographic emulsion (Kodak NTB3) (*8*). The autoradiograms were exposed for 75 to 150 days, then photographically processed and stained with methyl green pyronine.

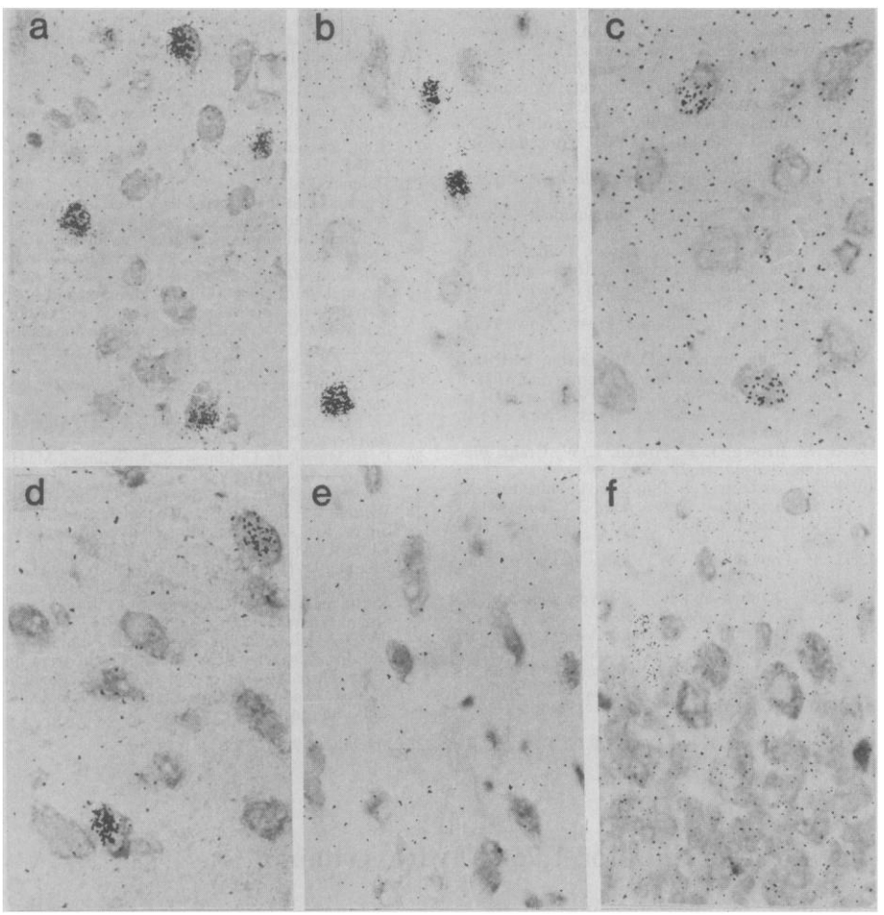

Fig. 1. Autoradiograms of rat brain showing nuclear concentration of radioactivity 2 hours after injection of ^{3}H-labeled $1,25(OH)_2D_3$ in neurons of the (a) nucleus centralis of the amygdala, (b) nucleus interstitialis of the stria terminalis, and (c) the outer zone of the nucleus spinalis caudalis of the trigeminus. In competition studies designed to chemically characterize the nuclear radioactivity, unlabeled substances were injected before treatment with labeled $1,25(OH)_2D_3$. (d) 25-OH-D_3 (nucleus centralis amygdalae) did not prevent nuclear uptake; (e) $1,25(OH)_2D_3$ (nucleus interstitialis striae terminalis) prevented nuclear uptake of radioactivity. (f) Neurons of the cerebellar cortex do not show nuclear labeling in the same animals that show labeling in (a to d). Scale bar, 4 μm; stain, methyl green pyronine. Magnification (a, b, e, and f), ×560; (c and d) ×880. Exposure time (a to c, e, and f), 150 days; (d), 75 days.

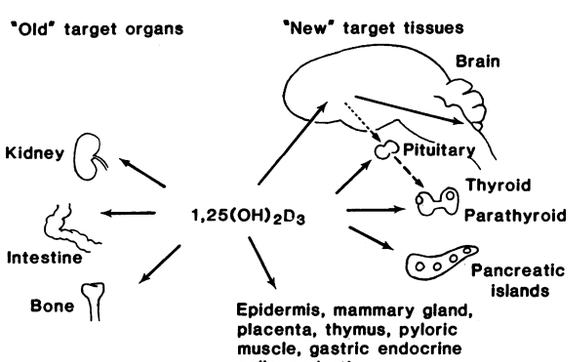

Fig. 2. Target cells for $1,25(OH)_2D_3$ (solid lines) have been identified by autoradiography, not only in the three classical target organs (left), but in many new target tissues (*6*) (right). Evidence for a vitamin D–related pituitary-thyroid functional connection (dashed line) has been obtained (*12*), and target cells for $1,25(OH)_2D_3$ are found in both neurons and pituitary cells. Therefore, a related brain-pituitary link is postulated (dotted line), and the existence of a neuroendocrine brain-pituitary-intestinal axis, which is involved in the central regulation of calcium homeostasis, is proposed. Like other steroid hormones, $1,25(OH)_2D_3$ may act on many tissues, some of which do not seem to be directly related to calcium metabolism, but involve other functions as well.

After the injection of ^{3}H-labeled $1,25(OH)_2D_3$, nuclear concentration and retention of radioactivity is seen in autoradiograms not only in the absorptive epithelial cells of the intestine (*1*) and in certain cells of the pituitary (*2*), but under the same conditions, in the brain and spinal cord (Fig. 1, a to f). Injection of $1,25(OH)_2D_3$, but not of 25-OH-D_3, before the injection of ^{3}H-labeled $1,25(OH)_2D_3$ abolished or diminished nuclear labeling. In the brain, the labeled cells were probably neurons, as judged by their size and location. In the forebrain, labeled cells were found in the nucleus centralis of the amygdala and in the rostrolateral portion of the bed nucleus of the stria terminalis. Nuclear labeling also occurred in neurons of the rostral thalamus, including the nucleus periventricularis, nucleus parataenialis, and nucleus rhomboides. Further nuclear labeling was found in the medulla oblongata in cells in the area postrema and its immediate vicinity in the nucleus tractus solitarii, and in scattered cells in the caudal spinal trigeminal nucleus. The labeled cells of the latter were continuous with labeled cells in the cervical spinal cord lamina II.

Among the labeled neurons, the strongest nuclear concentration of radioactivity was observed in the bed nucleus of the stria terminalis and the nucleus centralis of the amygdala. Neurons in the dorsal thalamus are weakly labeled in comparison with the other regions in the brain and the duodenum (*9*). The observation that the nuclear labeling in most brain regions is equal to or stronger than that in the duodenum suggests—in conjunction with the results of the competition studies—that the nuclear radioactivity represents $1,25(OH)_2D_3$.

The anatomical distribution of the target neurons in the brain indicates the existence of specific neuronal circuits for $1,25(OH)_2D_3$, similar to those proposed for estradiol (*10, 11*). The region involved includes a portion of the stria terminalis and a sensory relay system in the spinal cord dorsal horn, the spinal trigeminal nucleus, and the area postrema–nucleus tractus solitarii. The stria terminalis is a neural pathway that interlinks brain regions related to olfaction and reproduction, including certain amygdaloid and preoptic-hypothalamic cell groups (*11*). Target neurons for

$1,25(OH)_2D_3$ in the central amygdaloid nucleus, in the bed nucleus of the stria terminalis, and in the anterior thalamus are located within projection fields of the stria terminalis, of which they seem to form a subsystem. An analogy to relationships between other steroid hormones and brain-endocrines is suggested (Fig. 2), and $1,25(OH)_2D_3$-related peptidergic messengers and links to the portal system in the median eminence and to pituitary secretion can be expected. The finding of $1,25(OH)_2D_3$ in the brain, the previous demonstration of $1,25(OH)_2D_3$ in the pituitary (*1, 2*), and the effects of this hormone on thyroid-stimulating hormone secretion (*12*) suggest central modulation of calcium homeostasis involving a brain-pituitary-thyroid axis (Fig. 2). The possibility of a brain-intestine link in endocrine regulation must also be considered, since $1,25(OH)_2D_3$ apparently acts on structures in the amygdala, which, when lesioned or electrically stimulated, produce changes in gastric motility and secretion (*13*).

Vitamin D–inducible calcium-binding protein has been found not only in many peripheral target tissues for $1,25(OH)_2D_3$ but also in cell bodies, dendrites, and axons of Purkinje cells of chicken brain (*14*). We did not observe a concentration of radioactivity in these cells in our present studies (Fig. 1f). Although a correspondence between $1,25(OH)_2D_3$ target cells and cells containing vitamin D–dependent calcium-binding protein seems to exist—at least in some tissues—such a correspondence needs to be established through the use of our autoradiography-immunohistochemistry technique (*15*). This technique permits the simultaneous visualization of radioactivity and antibodies in the same preparation. Clinical reports indicate the importance of vitamin D action for normal brain function. In humans, retardation of mental growth has been observed in both vitamin D hypervitaminosis and hypovitaminosis (*16*). The frequency of epileptic seizures was reduced after vitamin D treatment, and this effect was unrelated to changes in serum calcium and magnesium (*17*). In hypoparathyroidism and pseudohypoparathyroidism, conditions in which $1,25(OH)_2D_3$ serum levels are low, seizures are a frequent symptom (*18*). Facilitated by the present anatomical data, the nature of the effects can now be studied and it can be clarified to which degree general or local actions of the hormone are involved.

$1,25(OH)_2D_3$ may act to maintain normal brain functions by providing normal serum calcium and phosphate levels. In addition, it may selectively affect specific neuronal populations with changes in metabolism, neuritic growth, alterations of sensitivity thresholds, and changes in production and secretion of certain aminergic-peptidergic messengers, as has been demonstrated for other steroid hormones.

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Intraspecific Vertical Stratification as a Mate-Finding Mechanism in Tropical Cockroaches

Abstract. *Cockroaches in a tropical forest stratify vertically both inter- and intraspecifically along micrometeorological gradients. At night, low wind speeds and unstable atmospheric conditions result in efficient vertical mixing of the air near the ground. Convective ascent of warm air imparts directionality to the pheromone-dispersion process. The occurrence of males at greater heights than pheromone-emitting conspecific females appears to be a mate-finding strategy.*

For airborne chemicals to be effective signals in communication, their release and reception must correlate with favorable micrometeorological conditions. To maximize their efficiency in finding pheromone-emitting females, males should occupy ranges above, overlapping, or below the females' vertical ranges, depending upon the prevailing atmospheric conditions. Since meteorological patterns exhibit circadian cycles, they couple the orientation behavior to specific times during the day or night. If, for example, buoyant (*1*) atmospheric conditions prevail at night, males should occupy perches at greater heights than females. Conversely, under stable (*1*) atmospheric conditions with temperature inversion, males should overlap with the vertical distribution of the females or range below them.

Dispersion models (*2*) describe plumes

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