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β-Adrenergic Receptor Involvement in 6-Hydroxydopamine-Induced Supersensitivity in Rat Cerebral Cortex

Abstract. *The intraventricular administration of 6-hydroxydopamine, a procedure which destroys noradrenergic nerve terminals in the central nervous system, caused an increase in the density of β-adrenergic receptors in rat cerebral cortex, without affecting their affinity for isoproterenol. The results suggest that changes in the density of adrenergic receptors are involved in 6-hydroxydopamine-induced supersensitivity at central noradrenergic synapses.*

Interaction of the neurotransmitter norepinephrine (NE) with β-adrenergic receptors leads to activation of the enzyme adenylate cyclase (E.C. 4.6.1.1) and to an increase in the intracellular production of adenosine 3′,5′-monophosphate (cyclic AMP) (*1*). Denervation of peripheral organs results in an enhanced response to exogenous NE involving both pre- and postsynaptic components (*2*). The former component reflects the loss of the presynaptic nerve terminals and the associated NE uptake system. Injection of 6-hydroxydopamine (6-OHDA) into the lateral ventricle causes a specific degeneration of central catecholaminergic nerve terminals and a depletion of catecholamines (*3, 4*). Noradrenergic neurons innervate the cerebral cortex and play an important role in regulating many behavioral states. It is therefore important to study the effect of denervation on the properties of these synapses.

In slices of rat cerebral cortex, activation of either α- or β-adrenergic receptors leads to an increased production of cyclic AMP (*5, 6*). Administration of 6-OHDA increased the production of cyclic AMP in response to activation of both types of receptors (*7, 8*). To characterize the molecular basis for this increase in responsiveness, several components of the β-adrenergic receptor–adenylate cyclase system were studied in rats that had been treated with 6-OHDA. Isoproterenol-stimulated accumulation of cyclic AMP was determined in slices of rat cerebral cortex. In the same experiments, the properties and density of β-adrenergic receptors were determined by using a potent β-adrenergic receptor antagonist, ^{125}I-labeled hydroxybenzylpindolol (HYP) (*9, 10*), as a radioactive ligand. Only the response mediated by β-adrenergic receptors was investigated in this study. Isoproterenol is a specific β-adrenergic receptor agonist, and ^{125}I-labeled HYP binds specifically to β-adrenergic receptors (*9, 10*).

Male Sprague-Dawley rats (120 to 160 g) were injected intraventricularly (*3, 11*) on each of two successive days with 200 μg (free base) of 6-OHDA (Regis Chemical) dissolved in 20 μl of 0.9 percent saline containing sodium ascorbate (1 mg/ml, *p*H 5). Controls were injected with 20 μl of vehicle. This dose of 6-OHDA decreased NE levels in the cerebral cortex by 81 ± 3 percent (N = 21). Rats were killed by decapitation 7 to 9 days after the first injection. The entire cerebral cortex from each rat was dissected free of midbrain structures, sliced (1 by 0.26 by 0.26 mm) with a McIlwain tissue chopper and resuspended in 15 ml of oxygenated (95 percent O_2 and 5 percent CO_2) Krebs-Ringer buffer (*5, 12*).

Approximately 20 percent of each resuspended cortex was homogenized in 15 ml of 0.32M sucrose, 10 mM tris, *p*H 7.5, by means of a motor-driven Teflon-glass homogenizer. These homogenates were centrifuged at 20,000g for 10 minutes. The resulting pellets were resuspended (200 ml per gram of original wet weight) in 0.9 percent NaCl, 20 mM tris, *p*H 7.5, for use in binding studies which were performed as previously described (*10*). The remainder of the chopped tissue was used to measure isoproterenol-induced cyclic AMP accumulation. A modification (*5*) of the method of Shimizu *et al.* (*12*) was utilized to follow the conversion of tritiated adenosine triphosphate (ATP) to tritiated cyclic AMP. For these experiments the tissue ATP pools were first labeled by incubating the slices for 30 minutes with tritiated adenine (2.5 μc/ml of resuspended tissue). The concentration-dependent effects of isoproterenol on the conversion of tritiated ATP to tritiated cyclic AMP were then followed. Isobutylmethylxanthine (1 mM), an inhibitor of phosphodiesterase (E.C. 3.1.4.1), was included in these experiments. Results are expressed as the percentage conversion of tritiated ATP to tritiated cyclic AMP (*12*). Concentrations of NE (*13*) and protein (*14*) were determined as previously described.

The accumulation of cyclic AMP in response to a maximally stimulating concentration of isoproterenol was 80 ± 5 percent (N = 13) greater in the 6-OHDA treated rats than in the controls (Fig. 1). Treatment of animals with 6-OHDA did not affect the concentration of *l*-isoproterenol needed to produce a half-maximal increase (ED_{50}) in the accumulation of cyclic AMP in slices of cerebral cortex (Fig. 1, inset). The ED_{50} determined from the data presented in Fig. 1 together with the data from two similar experiments was 33 ± 1 nM (N = 13) for controls and 34 ± 2 nM (N = 14) for treated rats.

The binding of ^{125}I-labeled HYP to a particulate fraction derived from rat cerebral cortex has properties similar to those which would be expected of binding to β-adrenergic receptors in vitro. The binding is reversible, saturable, of high affinity, stereospecific, and it is inhibited by appropriate β-adrenergic receptor ligands (*10*). Specific binding (approximately 80 percent of the total ^{125}I-labeled HYP binding) was defined as binding which was inhibited by 0.3 μM *dl*-propranolol. The density of β-adrenergic receptors in the cerebral cortex of 6-OHDA treated and control rats was determined by measuring the specific binding of various concentrations of ^{125}I-labeled HYP and analyzing the data by the

method of Scatchard (*15*). The results are consistent with the idea that there is only a single class of high affinity binding sites. There was a 31 percent increase ($P < .001$) in the concentration of ^{125}I-labeled HYP binding sites in the cortex of rats which had been treated with 6-OHDA (Table 1). The dissociation constants (K_d) of ^{125}I-labeled HYP and of *l*-isoproterenol were determined by Scatchard analysis and by inhibition of the binding of ^{125}I-labeled HYP (*9, 10, 16*), respectively. Administration of 6-OHDA did not change the K_d value for either compound. The binding of ^{125}I-labeled HYP in both control and 6-OHDA treated rats showed neither positive nor negative cooperativity (Hill coefficients ranged from 0.95 to 1.05). Since isoproterenol is only minimally affected by the presynaptic uptake system (*17*) the increased accumulation of cyclic AMP is unlikely to have been caused by the degeneration of the presynaptic terminals. Similar findings have been reported in studies of NE- and isoproterenol-stimulated cyclic AMP accumulation in brain slices following the administration of 6-OHDA (*7, 8*) or reserpine (*18*). In each of these studies an increase in the efficacy of catecholamines was seen as a consequence of postsynaptic changes.

The effect of 6-OHDA treatment on the maximal accumulation of cyclic AMP (80 percent increase) was greater than its effect on the density of β-adrenergic receptors (31 percent increase). The disparity between the absolute magnitude of the increase in cyclic AMP production and the increase in the density of β-adrenergic receptors suggests that denervation may have multiple effects which bear on the responsiveness of the system. Kalisker *et al.* (*8*) reported that the administration of 6-OHDA had no effect on fluoride-stimulated adenylate cyclase activity in homogenates of rat cerebral cortex. However, in the present experiments a consistent (20 to 30 percent) increase in basal levels of cyclic AMP accumulation was observed in the 6-OHDA treated animals (Fig. 1). This may suggest that there is a small increase in enzyme molecules after the administration of 6-OHDA, which could explain part of the discrepancy noted above.

Isoproterenol was approximately nine times more potent in stimulating cyclic AMP formation in slices than in inhibiting ^{125}I-labeled HYP binding to a particulate fraction. The difference in the tissue preparation does not appear to be responsible for the discrepancy in the potency of isoproterenol in the two systems since it was also observed when binding studies were performed with cortical slices. This preparation was not used routinely for binding studies since specific binding was only 30 to 50 percent of total binding.

Table 1. Affinities of ^{125}I-labeled HYP and *l*-isoproterenol for β-adrenergic receptors, and the density of β-adrenergic receptors in the cerebral cortex of control and 6-OHDA treated rats. The dissociation constant (K_d) of ^{125}I-labeled HYP and the density of binding sites (B_{max}, expressed as picomoles per milligram of protein) were determined by Scatchard analysis (*10, 15*), and the K_d of *l*-isoproterenol was determined by measuring the ability of *l*-isoproterenol to inhibit the binding of ^{125}I-labeled HYP. The concentration required to inhibit binding by 50 percent is taken to be the K_d of *l*-isoproterenol (*10, 16*). Each value is the mean ± standard error of independent determinations on preparations from 13 to 19 animals (numbers shown in parentheses).

Treatment	K_d		B_{max} (pmole/mg)
	^{125}I-labeled HYP (n*M*)	*l*-isoproterenol (μM)	
Control	1.1 ± 0.09 (17)	0.27 ± 0.02 (14)	0.29 ± 0.01 (19)
6-OHDA	1.2 ± 0.07 (17)	0.31 ± 0.05 (13)	0.38 ± 0.01 (17)*

*$P < .001$ when compared to control value (two-tailed test).

Recent results from our laboratory (*19*) indicate that a critical factor in the ontogeny of catecholamine-induced cyclic AMP accumulation in rat cerebral cortex is the appearance of β-adrenergic receptors. Similarly, increases in β-adrenergic receptors and catecholamine-responsive adenylate cyclase activity have been observed in the pineals of rats kept in constant light for 24 hours (*20*) and in the livers of adrenalectomized rats (*21*). The present results suggest that the regulation of the postsynaptic response to catecholamines at central noradrenergic synapses is mediated at least in part by changes in the density of β-adrenergic receptors. The effect of denervation on receptor density is thus similar to that which has been studied after denervation of skeletal muscle (*22*) or parasympathetic ganglia (*23*).

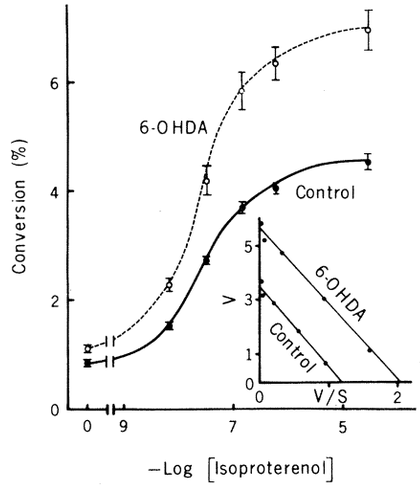

Fig. 1. Effect of 6-OHDA on isoproterenol-stimulated cyclic AMP accumulation in slices of rat cerebral cortex. Slices were labeled with tritiated adenine and then incubated with varying concentrations of isoproterenol as described in the text. The ordinate represents the percentage of tritiated ATP which was converted to tritiated cyclic AMP. The inset shows the same data plotted as described by Hofstee and by Eadie (*24*). The percentage of tritiated ATP converted to tritiated cyclic AMP in the presence of a known concentration of isoproterenol minus the percentage converted in the absence of isoproterenol (V) is plotted against V divided by the isoproterenol concentration (V/S). The results are for one experiment with eight control and eight treated rats and are representative of three similar experiments. The K_d values for this experiment, derived from the slopes of the lines, were 30 n*M* for the controls and 28 n*M* for the rats treated with 6-OHDA.

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Oldest Horse Brains: More Advanced Than Previously Realized

Abstract. *Previous interpretations of early horse brains were based on an incorrectly identified fossil endocast, now believed to be from a condylarth. Newly prepared endocasts of* Hyracotherium, *the oldest horse and one of the earliest perissodactyls, reveal a relatively larger brain, with a more expanded neocortex, than existed in the condylarth ancestors of perissodactyls. Fifty million years ago, horse brains had suprasylvian, ectolateral, and lateral sulci, but the frontal lobe was undeveloped.*

Almost 30 years ago, Edinger (*1*) described the brain of Eohippus (*Hyracotherium*), the oldest horse, as being more primitive than that of any living placental mammal and strikingly similar to that of *Didelphis*, the marsupial opossum. That description was important because *Hyracotherium* was the earliest horse and also one of the earliest members of the order Perissodactyla (horses, rhinos, tapirs, and some extinct families). Therefore, knowledge of its brain morphology has relevance for understanding the factors involved in the origin of the order, and also for interpreting subsequent brain evolution in perissodactyls.

Edinger's interpretation of the brain of *Hyracotherium* was based largely on a natural stone endocast with only ventral braincase bones attached (Yale Peabody Museum 11694) from the early Eocene (about 50 to 55 million years ago) of New Mexico. However, there are no teeth or other diagnostic elements that allow definite identification of YPM 11694 as *Hyracotherium* (or as any other perissodactyl). Further, the optic foramen is smaller and more rostrally located in YPM 11694 than in known *Hyracotherium* specimens (*2*), which indicates that YPM 11694 has been incorrectly identified. To see what the brain of the earliest horses actually looked like, I prepared endocasts from three skulls with dentitions, unequivocably identified as *Hyracotherium* (*3*). These endocasts reveal that the brain was considerably advanced, in terms of neocortical expansion, over the condition described by Edinger, and also advanced compared to brains of the archaic ungulates from which the order Perissodactyla arose.

The new *Hyracotherium* endocasts (Fig. 1, A and B), show that the brain had three neocortical sulci. The longest and most prominent sulcus lay immediately dorsal to the rhinal fissure and extended rostrocaudally for most of the length of the cerebrum. Dorsal to the caudal portion of that sulcus lay two shorter and less marked sulci. Comparisons with later perissodactyl endocasts, which reveal the evolutionary history of neocortical folding in the order (*1*, *4*), suggest that the sulci indicated on the *Hyracotherium* endocasts represent, from ventral to dorsal, the suprasylvian, ectolateral, and lateral sulci of modern perissodactyls. A shallow notch in the rhinal fissure, which delimited a rostral one-fifth of the cerebrum, marks the later (future) position of the sylvian sulcus. The caudal portion of the rhinal fissure was oriented dorsocaudally, and was overlain by a vascular sinus.

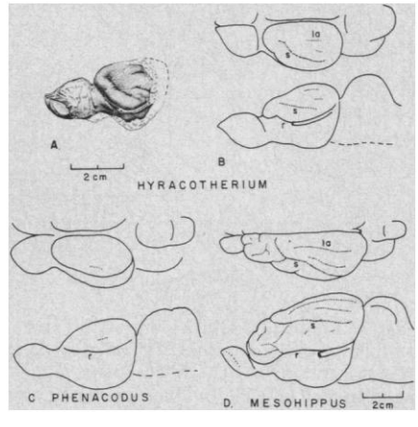

Fig. 1. Fossil endocasts. (A) Oblique view of slightly crushed endocast of forebrain of *Hyracotherium*, a 50-million-year-old horse, American Museum of Natural History (AMNH) No. 55267. (B) Dorsal and lateral views of *Hyracotherium* endocast, composite based on AMNH Nos. 55266, 55267, and 55268. (C) Endocast of *Phenacodus*, a condylarth contemporary of *Hyracotherium*, based on AMNH No. 4369 and Museum of Comparative Zoology No. 4440. (D) Endocast of *Mesohippus*, a 30-million-year-old horse, AMNH No. 9814. Abbreviations: *la*, lateral sulcus; *r*, rhinal fissure; *s*, suprasylvian sulcus; (A, B, and C) slightly more than one-third size; (D) slightly more than one-fourth size. The scales are 2 cm.

A short gap separated the rostral pole of the cerebrum from the olfactory bulbs, which were pedunculate. The olfactory bulbs were large relative to the rest of the brain. The caudal pole of the cerebrum appears to have extended back almost to the cerebellum, and little if any of the midbrain was exposed dorsally. The cast of the cerebellum was not preserved well enough to reveal details beyond gross size and shape.

The *Hyracotherium* endocasts described above are from a species that lived in the latter part of the early Eocene, about 50 million years ago, about 10 million years after the origin of the order. Perissodactyls show little evidence of evolutionary change during the early Eocene, and the oldest known specimens of *Hyracotherium*, which are also the oldest known perissodactyls, differ little in known morphology (cheek teeth) from the later *Hyracotherium* species whose endocasts are here described (*5*). Nevertheless, because of the amount of elapsed time, it is possible that the available *Hyracotherium* endocasts represent a brain morphology somewhat more advanced than that of the earliest perissodactyls. We must await the discovery of additional fossil endocasts to learn whether the brain morphology here described also characterized that of the earliest perissodactyls.

The fossil record suggests that perissodactyls arose in the late Paleocene (about 60 million years ago), from a group of archaic ungulates, the phenacodontid condylarths (*6*). The only phenacodontid endocasts known are from *Phenacodus*, a genus that survived through the early Eocene, and coexisted with *Hyracotherium* and other early perissodactyls before becoming extinct. The brain of *Phenacodus* (Fig. 1C) was more primitive than that of *Hyracotherium* in having a less expanded neocortex: note the higher rhinal fissure, fewer neocortical sulci, and less expanded frontal