Perinatal Undernutrition Reduces Alpha and Beta Adrenergic Receptor Binding in Adult Rat Brain

Abstract. *Specific alpha and beta receptor binding is significantly reduced in the brains of adult rats that were undernourished perinatally. From kinetic studies it may be concluded that this effect is the result of a diminished number of binding sites, not changes in receptor affinity.*

Undernutrition in early life produces anatomical, neurological, neurochemical, and behavioral alterations (*1*). Changes in the levels and metabolism of biogenic amines have also been described (*2*). Marichich *et al.* (*3*) recently reported that rats, fed a low-protein diet between the last week of pregnancy and 50 days of age and then given balanced laboratory feed for 90 days, showed an enhanced rate of brain catecholamine turnover and a significant increase in brain tyrosine hydroxylase activity. This suggests that neurotransmitters are released at a higher rate in rats undergoing such treatment. Since increased neuronal activity may induce changes in postsynaptic receptors, we studied alpha and beta receptor binding in the brains of adult rats that were undernourished perinatally. Our results indicate a decrease in the number of binding sites without any apparent change in the affinity for the ligand.

Female rats derived from a Wistar strain were divided into two groups at 14 days of pregnancy and fed isocaloric diets containing 24 or 8 percent casein (*3*). After weaning (24 days), the pups continued consuming the same diet as their dams until they reached 50 days of age. Thereafter both groups were given balanced laboratory feed for at least 90 days.

Adrenergic binding assays were performed in accordance with the method of Alexander *et al.* (*4*). Whole brain homogenates, prepared with a Teflon pestle in 5 to 8 volumes of cold buffer (0.21*M* sucrose, 5 m*M* tris-HCl, and 1 m*M* $MgCl_2$; *p*H 7.4), were centrifuged for 20 minutes at 800*g* and 4°C. The pellets were discarded, and the supernatants were centrifuged for 14 minutes at 14,000*g* and 4°C. The resulting pellets were washed once in the same volume of sucrose buffer and resuspended in cold buffer (75 m*M* tris-HCl and 25 m*M* $MgCl_2$; *p*H 7.4) to a final protein concentration of 200 to 300 μg per 100 μl. Proteins were determined by the method of Lowry *et al.* (*5*). Tritiated dihydroergocryptine (DHK) and tritiated dihydroalprenolol (DHA) were used as ligands in the determination of alpha and beta receptors, respectively. For total binding each tube received 100 μl of the membrane suspension and 5 μl of a dilution of DHK or DHA. The tubes were incubated for 30 minutes at 25°C (alpha receptors) or for 10 minutes at 37°C (beta receptors). For nonspecific binding the tubes contained in addition 5 μl of phentolamine or DL-propranolol (to a final concentration of $1.10^{-5}M$) in the assay of alpha and beta receptors, respectively. In both cases the final volume was 110 μl. The incubation was terminated by adding 2.5 ml of cold buffer to each tube and rapidly filtering the contents under reduced pressure through Whatman GF/B filters. The incubation tubes were rapidly washed twice with 2.5 ml of cold buffer and the filters were dried and transferred to vials to count the radioactivity in a fluid that contained Triton X-100 and toluene. Specific binding was defined as the difference in radioactivity in the absence and presence of unlabeled ligands. The assays were performed in triplicate.

Scatchard plots made after linear regression analysis of the data show fewer binding sites in the experimental rats than in the controls. The decrease involved both alpha (Fig. 1A) and beta (Fig. 1B) receptors. In malnourished animals the maximal number of binding sites was reduced 27 and 35 percent for alpha and beta receptors, respectively. The effects on ligand affinity and receptor density are summarized in Table 1 for alpha receptors and in Table 2 for beta receptors. In both cases there was no significant difference between dissociation constants.

Treatments that depress the presynaptic activity of catecholaminergic neurons induce supersensitivity, while those that increase neuronal activity produce subsensitivity. Thus, such drugs as 6-hydroxydopamine, which causes degeneration of adrenergic terminals, and reserpine, which blocks neuronal storage, induce postsynaptic supersensitivity (*6*). Conversely, tricyclic antidepressants, which block neuronal uptake, and amphetamine, which increases the release of neurotransmitters, induce subsensitivity (*7*). These changes in sensitivity are attributed to modifications in the number of binding sites (*8*). We found that adult

Table 1. Specific binding of DHK to alpha receptors. Values are means ± standard errors.

Group	*N*	Dissociation constant	Maximum binding (fmole/mg protein)
Control	5	24.4 ± 3.8	636.4 ± 32.3
Deprived	5	23.3 ± 4.7	467.2 ± 41.6*

*$P < .02$ (Student's *t*-test).

Table 2. Specific binding of DHA to beta receptors. Values are means ± standard errors.

Group	*N*	Dissociation constant	Maximum binding (fmole/mg protein)
Control	7	32.0 ± 4.6	323 ± 28.7
Deprived	7	23.2 ± 2.5	210 ± 25.1*

*$P < .02$.

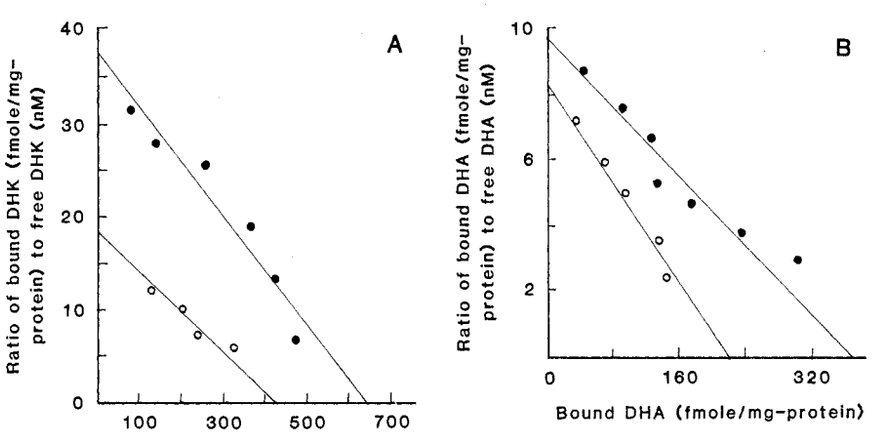

Fig. 1. Scatchard plots of data from representative experiments, showing reductions in receptor binding in brains of experimental animals (○) compared with controls (●). The labeled ligands were used in concentrations varying from 5 to 50 n*M* (DHK) and 3 to 30 n*M* (DHA).

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rats nutritionally deprived during their perinatal period and then afforded prolonged dietary rehabilitation demonstrate a significant decrease in the number of alpha and beta adrenergic receptors in the brain. No apparent change in the affinity for either ligand (DHK or DHA) was detected. Such an effect may be a consequence of permanent activation of central noradrenergic neurons inducing greater release of neurotransmitters in the recovered adults, and may be interpreted as the result of postsynaptic adaptations intended to balance the neuronal hyperactivity. The decrease in the number of adrenergic receptors as a consequence of perinatal undernutrition may induce changes in the reactivity to drugs whose mechanisms of action involve the central catecholaminergic system.

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References

1. O. Resnick, M. Miller, W. Forbes, R. Hall, T. Kemper, J. Bronzino, P. J. Morgane, *Neurosci. Biobehav. Rev.* **3**, 233 (1979); A. Ogura and O. A. Orsingher, *Behav. Neurol. Biol.* **28**, 105 (1980); W. C. Stern, W. Forbes, O. Resnick, P. J. Morgane, *Brain Res.* **79**, 375 (1974); S. Zammenhof, E. van Marthens, F. L. Margolis, *Science* **160**, 322 (1968); R. M. Barnes, S. R. Cunnold, R. R. Zimmermann, H. Simmons, R. B. Mac Lead, L. Krook, *J. Nutr.* **89**, 399 (1966); J. L. Smart, *Psychiatr. Neurol. Neurochir.* **74**, 443 (1971).
2. W. J. Shoemaker and R. J. Wurtman, *Science* **171**, 1017 (1971); *J. Nutr.* **103**, 1537 (1973); C. Lee and R. Dubos, *J. Exp. Med.* **163**, 1031 (1972); T. J. Sobotka, M. P. Cook, R. E. Brodie, *Brain Res.* **65**, 443 (1974).
3. E. S. Marichich, V. A. Molina, O. A. Orsingher, *J. Nutr.* **109**, 1045 (1979).
4. R. W. Alexander, J. N. Davis, R. J. Lefkowitz, *Nature (London)* **258**, 437 (1975).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
6. K. Dismukes and J. W. Daly, *Mol. Pharmacol.* **10**, 933 (1974); G. C. Palmer, *Neuropharmacology* **11**, 145 (1972); A. Kalisker, C. O. Rutledge, J. P. Perkins, *Mol. Pharmacol.* **9**, 619 (1973).
7. M. P. Martres, M. Baudry, J. C. Schwartz, *Nature (London)* **255**, 731 (1975); A. Frazer, G. Pandey, J. Mendels, S. Neeley, M. Kane, M. E. Hess, *Neuropharmacology* **13**, 1131 (1974); J. Vetulani *et al.*, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **293**, 109 (1976).
8. J. R. Sporn, T. K. Harden, B. B. Wolfe, P. B. Molinoff, *Science* **194**, 624 (1976); S. R. Nahorski, *Mol. Pharmacol.* **13**, 679 (1977); S. P. Banerjee, L. S. Kung, S. J. Riggi, S. K. Chanda, *Nature (London)* **268**, 455 (1977); J. Schwartz, J. Costentin, M. Martres, P. Protais, M. Baudry, *Neuropharmacology* **17**, 665 (1978).

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The Regulation of Infanticide and Parental Behavior: Implications for Reproductive Success in Male Mice

Abstract. *Infanticide has been proposed to be a pathological response to overcrowding or other forms of environmental stress and thus a maladaptive behavior. However, in male house mice this behavior is predictable and is modulated by learning. Committing infanticide can increase a male's reproductive success and in some situations may therefore be an adaptive behavior.*

Infanticide, the killing of young, occurs in many species, including humans (*1*). Experiments with rodents on the effects of overcrowding on social behavior led to the hypothesis that infanticide is a response to a breakdown in social structure and, as such, is a pathological or nonadaptive behavior (*2*). Recently, it was suggested that in some circumstances an animal may benefit from committing infanticide (*3*). Infanticide is thus considered to have evolved in response to positive selective pressure and to be predictable and adaptive. Hrdy (*3*) classified infanticide in terms of the manner in which an animal might benefit from killing infants. Our experiments concern one category of infanticide—that relating to sexual competition between males.

It has been suggested that a male that commits infanticide can increase his reproductive success at the expense of competitors by killing a competitor's offspring and then mating with the mother. This sexual-competition hypothesis is based on Darwin's concept of sexual selection and involves three assumptions: (i) that there exist mechanisms to assess paternity so that males are unlikely to kill their own offspring, (ii) that the killing of a female's young results in her ovulating and mating with the infanticidal male sooner than she could have had her young not been killed (ovulation being inhibited in a lactating female), and (iii) that the behavior is mediated at least in part by genotype and thus heritable. If these criteria are met, then once the genotype for infanticide appears, it should rapidly increase in frequency in a population, since males with the infanticidal genotype should produce more offspring than males with the noninfanticidal genotype (*3*). Our experiments were designed to test the sexual-competition hypothesis.

There is evidence that only the one dominant male mouse in a breeding group mates and produces young (*4*). Since dominance status appears to be a major factor in determining the reproductive success of a male mouse, the relation of dominance status to the behavior of male mice toward newborn young was examined. Studies have revealed that mating experience reduces the proportion of male mice that commit infanticide (*5*). Thus, the possibility that mating experience per se might serve as a mechanism for parental recognition and inhibit male mice from committing infanticide only during the time that they would be in contact with their own young (3 to 8 weeks after mating) was also examined.

Male mice (CF1) that were sexually naïve were paired, and males that had mated with two females 2 weeks earlier were also paired (*6*). Members of a pair were placed together for 1 hour each day for 7 days. Dominance was assessed by observing males fighting during the last day of pairing and by examining each animal's rate of urine marking the next day. Dominant male mice deposit hundreds of urine marks, but subordinate males excrete urine in a few large pools (*7*). Only animals that could be clearly classified as dominant or submissive in terms of both aggressiveness and urine marking were examined for infanticide on the day after urine marking. For the test two newborn mice (sired by other males) were placed in the corner of a male's cage for 30 minutes. A preliminary study had demonstrated that neither the sex nor the age of the young influences the tendency of male mice to commit infanticide (*8*). Three behaviors were recorded when the young were removed from a male's cage: (i) infanticide—one or both of the young were severely wounded or killed; (ii) parental behavior—one or both of the young were found in the nest with the male hovering over them; and (iii) ignored—neither newborn mouse was wounded or in the nest, and both young were cold (*9*). Most sexually naïve dominant males committed infanticide, and most sexually experienced dominant males exhibited parental behavior. Subordinate males, regardless of mating experience, tended to behave parentally (Table 1).

The test for infanticide was conducted 3 weeks after some of the dominant males had mated because the length of pregnancy in house mice is 19 to 21 days. To determine whether the inhibition of infanticide, which had been observed 3 weeks after mating in the dominant, sexually experienced males, was temporary, the dominant males ($N = 33$) were retested 3 months after they had mated with two females (9 weeks after the initial test for infanticide). On the retest,

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