antagonized by relatively high concentrations of the α-adrenergic antagonist phentolamine (*2, 13*), it seems likely that the dopamine stimulation of cyclase may involve a kind of α-adrenergic receptor or, perhaps, a dopamine receptor different from that of the adrenergic nerve terminal of the rabbit ear artery.

The inhibitory effect of dopaminergic agonists on neurotransmission in the rabbit ear artery represents a pharmacological model for quantitative studies on dopaminergic receptor mechanisms. Since both antagonist and agonist activity can be assayed, this model may prove useful for the development of new dopaminergic antagonists for the treatment of psychotic disorders, as well as dopaminergic agonists for the treatment of parkinsonism, a disease associated with depletion of striatal dopamine levels (*14*).

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References and Notes

1. J. M. Van Rossum, *Arch. Int. Pharmacodyn. Ther.* **160**, 492 (1966); G. N. Woodruff, *Comp. Gen. Pharmacol.* **2**, 439 (1971).
2. L. L. Iversen, *Science* **188**, 1084 (1975).
3. S. H. Snyder, S. P. Banerjee, H. I. Yamamura, P. Greenberg, *Science* **184**, 1243 (1974); S. S. Kety and S. Matthysse, *Neurosci. Res. Bull.* **10**, 370 (1972).
4. B. K. Koe, in *Neuroleptics*, S. Fielding and H. Lal, Eds. (Futura, New York, 1974), p. 131; J. L. Neumeyer, W. P. Dafeldecker, B. Costall, R. J. Naylor, *J. Med. Chem.* **20**, 190 (1977).
5. M. W. McCulloch, M. J. Rand, E. F. Story, *Br. J. Pharmacol.* **49**, 141P (1973); M. A. Enero and S. Z. Langer, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **289**, 179 (1975); M. Ilhan, J. P. Long, J. G. Cannon, *Arch. Int. Pharmacodyn. Ther.* **222**, 70 (1976); F. M. Sharabi, J. P. Long, J. G. Cannon, G. J. Hatheway, *J. Pharmacol. Exp. Ther.* **199**, 630 (1976).
6. O. S. Steinsland, R. F. Furchgott, S. M. Kirpekar, *J. Pharmacol. Exp. Ther.* **160**, 346 (1973); *Circ. Res.* **32**, 49 (1973).
7. S. Z. Langer, *Biochem. Pharmacol.* **23**, 1793 (1974); M. J. Rand, M. W. McCulloch, D. F. Story, in *Central Action of Drugs in Blood Pressure Regulation*, D. S. Davies and J. L. Reid, Eds. (University Park Press, Baltimore, 1975), p. 94; K. Starke, H. D. Taube, E. Borowski, *Biochem. Pharmacol.* **26**, 259 (1977).
8. K. Starke, T. Endo, H. D. Taube, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **291**, 55 (1975); O. S. Steinsland and S. H. Nelson, *Blood Vessels* **12**, 378 (1975).
9. O. S. Steinsland and J. P. Hieble, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 266 (1976).
10. P. Seeman, T. Lee, M. Chau-Wong, K. Wong, *Nature (London)* **261**, 717 (1976); I. Creese, D. R. Burt, S. H. Snyder, *Science* **192**, 481 (1976).
11. D. R. Burt, I. Creese, S. H. Snyder, *Mol. Pharmacol.* **12**, 800 (1976).
12. J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145 (1972).
13. A. S. Horn, in *Handbook of Psychopharmacology*, L. L. Iversen, S. D. Iversen, S. H. Snyder, Eds. (Plenum, New York, 1975), vol. 2, p. 179; J. W. Kebabian, Y. C. Clement-Cormier, G. I. Petzold, P. Greengard, *Adv. Neurol.* **9**, 1 (1975).
14. O. Hornykiewicz, *Br. Med. Bull.* **29**, 172 (1973).
15. R. F. Furchgott, *Annu. Rev. Pharmacol.* **4**, 21 (1964).
16. Butyrophenones and diphenylbutylpiperidines were donated by McNeil and Janssen, butaclamol by Ayerst, and perphenazine by Schering. Supported in part by a Pharmaceutical Manufacturers Association Foundation Research Starter Grant.

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Maternal Behavior as a Regulator of Polyamine Biosynthesis in Brain and Heart of the Developing Rat Pup

Abstract. *Rat pups removed from the mother and placed in a warm incubator for 1 hour or more show a 50 percent reduction in ornithine decarboxylase activity in the brain and heart. The decline is not caused by lack of nutrition. Instead, these studies suggest that active maternal behavior is necessary to maintain normal polyamine metabolism in brain and heart of the pup during development.*

Data from various mammalian species suggest that early interruption of mother-infant interaction may have biochemical, physiological, and behavioral consequences (*1*). Ornithine decarboxylase (ODC) (E.C. 4.1.1.17) is the first and probably rate-limiting step in polyamine biosynthesis, and it is elevated in tissues undergoing rapid growth and differentiation (*2*). In the brains and hearts of preweanling rats, a developmental pattern of ODC activity exists, with enzyme activity reaching a peak during periods of maximum DNA and RNA synthesis and returning to low levels as the period of rapid brain growth ends (*3*). Thyroxine and cortisol alter this developmental brain ODC activity pattern in a direction similar to the effects of these hormones on brain morphology, and protein synthesis, and behavioral development; it has been suggested that ODC activity is a sensitive index of maturation of the central nervous system (CNS) (*4*). We have reported that removal of rat pups from the mother prior to weaning for periods as short as 1 hour leads to a reduction of as much as 50 percent in brain and heart ornithine decarboxylase activity, as well as its immediate polyamine product, putrescine. The decline in ODC activity is not caused by alterations in the body temperature of the pups, since temperatures of deprived and nondeprived pups are the same, and the ODC activity is reversed rapidly by return to the biological mother or an accepting (lactating) foster mother (*5*).

Hofer (*6, 7*) has reported that a fast and readily reversible decrease in heart rate occurs in 14-day-old rat pups after they are removed from the mother. These alterations in heart rate have been shown to be related to nutrition (*6, 7*). The purpose of our experiments was to examine the role of nutrition and other factors in the maternal deprivation-induced decline in ODC activity in brain and heart tissue.

Gravid Sprague-Dawley rats (Zivic-Miller) were obtained 1 week before delivery, housed individually, and given free access to food and water. These rats delivered normally on day 21 of gestation. On the day of an experiment, pups were transferred from the vivarium to our laboratory where all subsequent studies were performed. For the maternal deprivation experiments, all pups were removed from the mother and either placed individually in a warm incubator or returned to the mother in the nest. At the end of an experiment, pups were decapitated, and ODC activity was determined by measuring the release of CO_2 from DL-[1-^{14}C]ornithine (43 mc/mole) (*8*), a reaction stoichiometric with the formation of putrescine (*3*). Enzyme activity was proportional to the tissue added and was linear for at least 1.5 hours. The cofactor, pyridoxal phosphate, at a concentration of 50 μM was used in all assays.

In the first set of experiments, lactating rats were anesthetized, and their nipples were ligated; they were then allowed to recover for 2 hours. Ten-day-old pups were then either placed with these mothers for 2 hours—the time twice that normally necessary to effect a decline in ODC activity induced by maternal deprivation—or allowed to remain with the biological mother. Pups placed with a lactating female whose nipples were ligated did not show a decrease in brain ODC activity (Table 1). In fact, a small but significant increase in enzyme activity occurred in these pups. Hofer and Weiner (*9*) have shown that pups placed with nipple-ligated lactating mothers spend more time with the mothers, presumably because the pups are continuously hungry. This may account for a slight increase in their brain ODC activity. These data suggest that

Table 1. Brain ODC activity of 10-day-old pups removed from the biological mother and placed for 2 hours either in a warm incubator or with a lactating foster mother whose nipples had been ligated. ODC activity is expressed as a percent of that of the nondeprived littermate controls.

Experimental condition of pups	*N*	ODC activity (% control)
With biological mother	44	100 ± 10
In incubator	28	24 ± 2*
With nipple ligated lactating female	45	140 ± 10*

*Significant difference from controls ($P < .05$, by Student's *t*-test).

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Table 2. Heart ODC activity of 10-day-old rat pups maternally deprived for 2 hours, deprived for 2 hours and returned for 2 hours, and deprived for 2 hours, injected with saline or propranolol (2 mg/kg in a volume of 0.5 ml) and returned for 2 hours. The ODC activity is expressed as a percentage of that of the controls.

Experimental conditions	N	ODC activity (% control)
Nondeprived		
No treatment	10	100 ± 10
Deprived		
No treatment	10	33 ± 2*
No injection and returned	11	114 ± 20†
Saline injection and returned	12	100 ± 10
Propranolol injection and returned	12	139 ± 16†

*Significant difference from control ($P < .05$, by Student's t-test or Newman-Keuls range test). †Not statistically different from control.

nutrition is not responsible for the decline in brain ODC activity as is the case with changes in heart rate.

Hofer (*7*) has shown that propranolol, a β-adrenergic antagonist, blocks the nutrition-mediated return of heart rate to baseline levels in 14-day-old rat pups that have been deprived and then returned to the mother. Also, propranolol has been reported to block β-adrenergic mediated increases in heart ODC activity (*7*). Therefore, the role of β-receptor stimulation in the return of heart ODC activity to baseline levels in pups deprived and then returned to their mother was examined. Pups were removed from the mother and home cage and kept in a warm incubator. After 2 hours away from the mother, pups were injected with 0.5 ml of propranolol (2 mg/kg) or 0.9 percent saline and returned to the mother. Two hours after being returned, pups were killed and ODC activity in the heart tissue was measured. Two hours of deprivation caused a 60 percent decrease in heart ODC activity, an effect which was reversed by return to the mother (Table 2). The reversal was not blocked by propranolol. Repeating the experiment with a large dose of propranolol (10 mg/kg) also failed to block the return in heart ODC activity. These data appear to exclude the possibility that the increase in ODC activity observed after the pups are returned to the mother is mediated through a nutrition-dependent neural mechanism, such as occurs with heart rate.

Apart from halting nutrition, maternal deprivation also interrupts suckling as well as active mothering behavior, such as licking and retrieving of pups. Since nutrition does not appear to be responsible for the deprivation-induced decline in ODC activity, interference with either of these other two aspects of the mother-pup interaction may be stressful to the pups, and in this way, initiate the decline in brain and heart ODC activity. To distinguish between these two possibilities, lactating female rats were anesthetized with urethane (1.1 g/mg). This treatment does not interfere with rat milk production and ejection or suckling (*10*), but obviously does eliminate active mothering behavior. When 10-day-old pups were placed for 2 hours with a urethane-anesthetized lactating female, the brain ODC activity was reduced about 55 percent (Table 3). This decline was the same as that caused by removal of pups from the mother. The possibility that urethane was crossing from mother to pup through the milk and thereby directly altering brain ODC activity was excluded by directly injecting pups with urethane (1.1 g/mg), waiting 2 hours, then killing the animals and measuring brain ODC activity; this treatment did not lower brain ODC activity (Table 3). These data suggest, therefore, that the absence of active mothering behavior and not suckling is responsible for the decrease in brain and heart ODC activity observed after maternal deprivation.

Serum glucocorticoids become elevated in response to many stressful stimuli (*11*) and, when administered exogenously, have been reported to cause numerous effects on brain growth and maturation, including the ability to inhibit ODC activity in preweanling rat brain (*4*). If adrenal glucocorticoids were involved in the maternal deprivation-induced decline in ODC activity, then adrenalectomy should block the effect. To test this possibility, 3-day-old pups were anesthetized lightly with ether, and both adrenal glands were removed through a single dorsal midline incision, which was then closed with suture. Control pups received the same anesthesia and operation except that the adrenal glands were left intact. All pups were then allowed to recover for 24 hours. Half of each litter was then maternally deprived for 2 hours, after which all pups were killed and brain ODC activity determined. As shown in Table 4, a 2-hour deprivation produced a similar decline in brain ODC activity in adrenalectomized and sham-operated controls, suggesting that maternal deprivation did not alter ODC activity through an adrenal mechanism.

Table 3. Brain ODC activity of 10-day-old rat pups. Group 1 rat pups were placed with anesthetized (urethane) or nonanesthetized mothers for 2 hours. Group 2 pups were injected intraperitoneally with urethane (1.1 g per kilogram of body weight) or 0.9 percent saline and returned to their lactating mothers. All pups were killed 2 hours after placement with the mother, and the ODC activity was determined. Brain ODC activity is expressed as a percentage of that of littermate controls.

Experimental condition of pups	N	ODC activity (% control)
Group 1		
With nonanesthetized mother	18	100 ± 13
With anesthetized mother	19	44 ± 4*
Group 2		
Injected with saline	11	100 ± 16
Injected with urethane	10	119 ± 15

*Significant difference from controls.

Table 4. Brain ODC activity of 4-day-old rat pups which were adrenalectomized or sham operated, allowed to recover for 24 hours, then maternally deprived in a warm incubator for 2 hours. ODC activity is expressed as a percent of the nondeprived littermate controls.

Experimental conditions	N	ODC activity (% control)
Not deprived		
Sham operated	9	100 ± 6
Adrenalectomized	10	100 ± 7
Deprived		
Sham operated	9	44 ± 4*
Adrenalectomized	9	57 ± 9*†

*Statistically significant difference from control ($P < .05$, by Student's t-test). †No significant difference from sham operated deprived pups.

In summary, these data demonstrate that maternal deprivation-induced decreases in brain and heart ODC activity, unlike deprivation-induced decreases in heart rate, are not mediated by nutrition, but instead appear to be caused by the absence of active mothering behavior. The adrenal hormones do not appear to mediate this response.

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References and Notes

1. M. A. Hofer, *J. Biol. Psychiatry* **10**, 149 (1975); H. F. Harlow and R. R. Zimmerman, *Science* **130**, 421 (1959); D. M. Levy, *Am. J. Orthopsychiatry* **4**, 203 (1934); P. F. D. Seitz, *Psychosom. Med.* **21**, 353 (1959).
2. D. Russell, *Polyamines in Normal and Neoplastic Growth* (Raven, New York, 1973); U. Bachrach, *Function of the Naturally Occurring Polyamines* (Academic Press, New York, 1973); A. Raina and J. Janne, *Med. Biol.* **53**, 121 (1975); C. W. Tabor and H. Tabor, *Annu. Rev. Biochem.* **45**, 285 (1976).
3. T. R. Anderson and S. M. Schanberg, *J. Neurochem.* **19**, 1471 (1972).

4. ———, *Biochem. Pharmacol.* **24**, 495 (1975).
5. S. R. Butler and S. M. Schanberg, *Trans. Am. Soc. Neurochem.* **8**, 259 (1977); *Life Sci.* **21**, 877 (1977).
6. M. A. Hofer, *Science* **168**, 871 (1970).
7. ———, *ibid.* **172**, 1039 (1971).
8. Radioactive ornithine was obtained from New England Nuclear.
9. M. A. Hofer and H. Weiner, *Psychosom. Med.* **33**, 353 (1971).
10. J. Bartolomé, C. Lau, T. Slotkin, *J. Pharmacol. Exp. Ther.*, in press.
11. D. W. Lincoln and J. B. Wakerley, *J. Physiol. (London)* **242**, 533 (1974).
12. G. Sayers, *Physiol. Rev.* **30**, 241 (1950).
13. This work was supported by NIH grant MH-13688 and NIH research scientist award MH-06489 to S.M.S.

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A Nonpeptide Morphine-Like Compound: Immunocytochemical Localization in the Mouse Brain

Abstract. *A nonpeptide morphine-like compound (MLC) which cross reacts with morphine-specific antibodies has been localized with the use of immunocytochemistry. This morphine-like compound is found in neuronal perikarya or processes (or both) in nuclei related to vestibular, cerebellar, and raphe systems.*

The nervous system contains endogenous compounds which in their actions resemble opiates (*1*). The most investigated of these compounds are the peptides such as enkephalin or endorphins. However, there is another type of endogenous morphine-like compound (MLC) which is not a peptide and which has been called MLC (*2*). In addition to binding to opiate receptors (*3*), MLC also binds to antibodies generated against morphine haptene (*2*). Critical to developing an understanding of the function of MLC is the determination of the type and distribution of the cells that contain it. We now report the localization of MLC in the perikarya and processes of neurons in nuclei related to vestibular, cerebellar, and raphe systems.

Rabbit antiserum containing antibodies directed specifically against morphine, first described by Spector (*4*), was used in these studies. Mice were killed by cervical dislocation, and the brains were removed and fixed for 4 hours in Bouin's reagent. The tissue was then dehydrated through a graded series of ethanol solutions, embedded in paraffin, and cut at 6 μm. Sections of fixed brain were then deparafinized, taken to water, and incubated with rabbit antiserum to morphine haptene for 72 hours in a humid atmosphere at 4°C. The antibody was localized by the unlabeled PAP (peroxidase-antibody to peroxidase) technique (*5*). For this technique, the sections were first incubated with the antiserum to morphine (diluted 1 : 500 in 0.1*M* phosphate buffered saline at *p*H 7.4), and rinsed twice in buffer for 5 minutes with constant shaking. The sections were then incubated (i) with sheep antiserum (diluted 1 : 100) to rabbit globulin for 30 minutes, (ii) treated by the PAP technique (antibody to peroxidase dilution, 1 : 200) for 1 hour, (iii) and then with 3,3′-diaminobenzidine (50 mg/100 ml) with 0.003 percent hydrogen peroxide for 6 minutes with agitation. The sections were rinsed in distilled water, dehydrated through a graded series of ethanol solutions and mounted in Permount. All solutions containing serums were passed through a Millipore (0.2 mm) filter just before use.

Controls consisted of serial sections to the ones exposed to specific antibody, but incubated instead with nonimmune serum, or morphine antiserum from which specific antibody had been removed by passage over a column containing morphine immobilized on Sepharose. No reactive neurons or processes were seen in these sections.

Several regions of the brain stem contained neurons reactive to MLC. One area showing intense immunoreactivity for MLC surrounded (within 0.2 mm) the floor of the caudal half of the fourth ventricle. There was much reaction product in neuronal perikarya and neurites in the medial vestibular nucleus and the praepositus hypoglossi (Fig. 1). Ependymal cells of the choroid plexus are not illustrated, but they also showed intense staining. Figure 2 shows staining of neuronal perikarya in the median raphe in the vicinity of the nucleus raphe magnus. Neurons and neuropil in the nucleus gigantocellularis also contained considerable reaction product (Fig. 3).

Other areas (not illustrated) in which reactive cells were seen included the vermis of the cerebellum, the deep cerebellar nuclei (for example, the dentate, fastigial, and interpositus, the nucleus of Roller, nucleus lateralis, medulla oblongata (*6*), and area postrema of the medulla.

Using techniques of light microscopic immunocytochemistry we have succeeded in localizing in both neuronal perikarya and processes a compound that cross reacts with specific antibody to morphine. The presence of this material in these structures suggests that it might play an important role in neuronal function. The presence of MLC in discrete nuclei further suggests that it might be associated with a restricted number of neuronal groups.

The localization of MLC in various brain stem nuclei could imply its involvement with certain neuronal pathways associated with stimulus produced analgesia (SPA). It has been well established that stimulation of medial brain stem structures such as periventricular structures and caudal raphe nuclei pro-

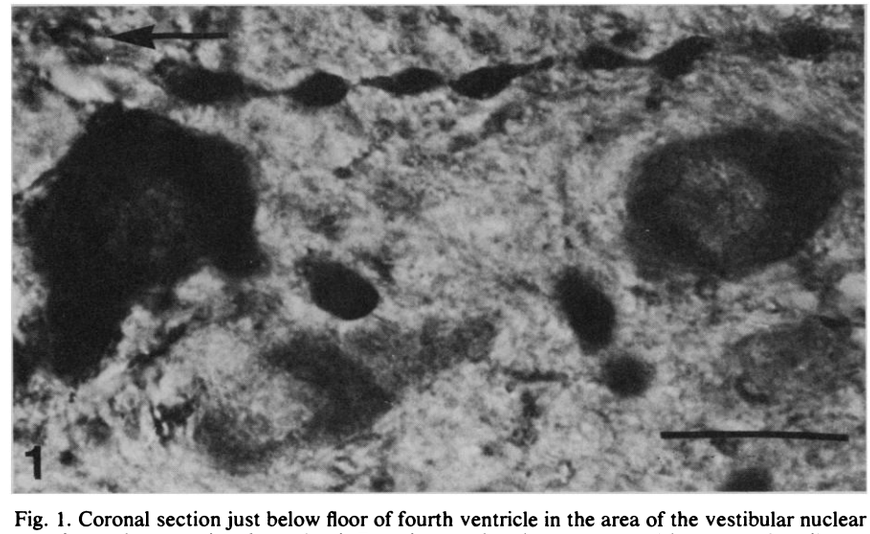

Fig. 1. Coronal section just below floor of fourth ventricle in the area of the vestibular nuclear complex and praepositus hypoglossi. Reaction product is concentrated in neuronal perikarya and in a varicose axon running across the upper portion of the field. Although reaction product is most intense in the varicosities, staining of thin intervaricose segments can also be seen. Arrow points in the direction of the fourth ventricle (scale bar, 20 μm).

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