Pharmacology Biochemistry & Behavior, Vol. 13, pp. 883–886. Printed in the U.S.A.

Neonatal Administration of Met-Enkephalin Facilitates Maze Performance of Adult Rats

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Received 15 October 1980

KASTIN, A. J.., R. M. KOSTRZEWA, A. V. SCHALLY AND D. H. COY. *Neonatal administration of Met-enkephalin facilitates maze performance of adult rats.* PHARMAC. BIOCHEM. BEHAV. 13(6) 883–886, 1980.—Newborn rats were injected SC during the first week of life with 80 μg/kg Met-enkephalin, DSIP, MIF-I, or diluent. When tested 3 months later in a 12-choice maze for a reward of food, hungry rats injected neonatally with Met-enkephalin were found to run the maze faster and with fewer errors than the controls. DSIP and MIF-I did not improve performance in the maze, indicating some specificity to the findings. Tyrosine hydroxylase and choline acetyltransferase activity in several parts of the brain were not significantly different among the groups. Radioimmunoassay of brain parts from a small number of adult rats indicated slightly more DSIP-like material in the thalamus and striatum of females injected neonatally with DSIP as compared with those injected with diluent. The results extend our previous observations of the persistence of central effects of peripherally injected Met-enkephalin from several minutes to three months when administration occurs early in life. The findings further suggest an effect of peptides on the organization of the developing brain.

Neonatal Maze Behavior Acquisition Appetitive Brain Infants Opiate Male Female

ADULT male rats injected peripherally with Met-enkephalin and tested in a complex maze were used to demonstrate the dissociation of behavioral and narcotic effects of opiate peptides in 1976 [9]. A relatively small dose (80 μg/kg body weight) was injected IP 15 min before hungry adult rats were placed in the 12 choice Warden maze. The use of this dose, route of administration, and time interval ruled out any analgesic effect. In the present study, rats were injected daily with the same dose of Met-enkephalin during the first week of life but not tested in the same system until 3 months of age.

Only a limited number of behavioral studies have been reported in adult rats injected neonatally with peptides. The peptides have included alpha-MSH [1,2], TRH [11], ACTH [5], an MSH/ACTH analog [3], and beta-endorphin [10]. Each peptide has been active in exerting a long-term effect, and no reports of inactive peptides have been published. The present study was designed to determine the specificity of this finding for several other peptides with rats of both sexes in a different test system.

METHOD

Apparatus

A complex maze consisting of 12 choice points leading toward a single goal box or blind alley was assembled from a series of galvanized metal straightaways and branched cul-de-sacs obtained from Lafayette Instruments Co. (Lafayette, IN). The following order was used: L (left), R, (Right), L, L, R, L, L, R, L, R, L, R. The food reward was made of a wet mash prepared with water daily from ground food pellets (Purina Laboratory Chow), powdered milk, and dextrose in a 4:4:1 ratio.

Animals

Seven pregnant female rats were obtained every two weeks for five weeks from Zivic Miller Co., Allison Park, PA. Newborn rats were injected SC in the dorsum of the neck once daily on days 1–7 of life. Members of each litter were randomly assigned to receive one of four treatments described below. Their ears were marked with India Ink. When separated by sexes at the time of weaning, their ears were also notched. They were housed under normal lighting conditions and with free access to food pellets for three months.

Treatment

The following three peptides were injected SC at a dose of 80 μg/kg (80 ng/g) body weight (usually a little more than 1 microgram/pup) for 7 days in a volume of 0.1 ml: Met-enkephalin, MIF-I (Pro-Leu-Gly-NH_2), DSIP. All dilutions were made with 0.01M acetic acid in 0.9% NaCl. This vehicle also served as the diluent control. Each solution was made fresh once a week for each batch of rats, coded so that the experimenter did not know the content, and kept at 4°C at all times.

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Procedure

After the first week of life, the rats were not handled by the experimenter until 90 days of age for the males and 97 days of age for the females. At that time, they were transferred from the animal quarters to a partitioned section of a sound-attenuated room in which the maze was housed. In this area, there was constant, indirect illumination and white background noise. The same procedure previously used for rats injected as adults with Met-enkephalin was used for these rats injected as infants [9]. Food deprivation to about 90% of body weight began at the time of transfer. After remaining in the new cages for 2 days, the rats were handled daily for the next 2 days. During the following 4 days, each rat was placed in the goal box containing food for 2 min each day. The maze was run for the final 4 days 15 minutes after IP injection with peptide or diluent. On the day of exploration as well as the last 3 days (designated as the days of acquisition), the rat was allowed to eat the mash for 1 min upon reaching the goal box. It was then transferred immediately to the start box for the second trial.

Enzyme Activity

Tyrosine hydroxylase activity was measured by the method of Waymire [13] and choline acetyltransferase activity by the method of Glover and Green [4]. These determinations were made in the striatum, cerebellum, frontal cortex, posterior cortex, and hypothalamus.

Radioimmunoassay of DSIP

DSIP-like material was measured by radioimmunoassay (RIA) in the brains of 5 adult male rats and 5 adult female rats injected with DSIP (80 μg/kg) during days 1–7 of life and in the brains of 5 adult male and 5 adult female rats injected with diluent during the same time. The previously described [7] method was modified by the use of chloramine-T for iodination and sonification of brain parts. At the time of assay the lyophoilized tissue was re-suspended at a concentration of 1.5 mg tissue/100 μl Tris buffer containing 0.1% human serum albumin and 6% Trasylol.

Statistics

The time that elapsed between the rat leaving the start box and reaching the food was considered the running time or latency. Each entry into a cul-de-sac was designated as an error. The calculations for running time and number of errors were based on the mean of the 2 trials for each day. Any mean greater than 500 seconds excluded the rat from all calculations. Differences among groups were determined from the raw data by two-way analysis of variance with repeated measures on the factor of days followed by Duncan's Multiple Range Test.

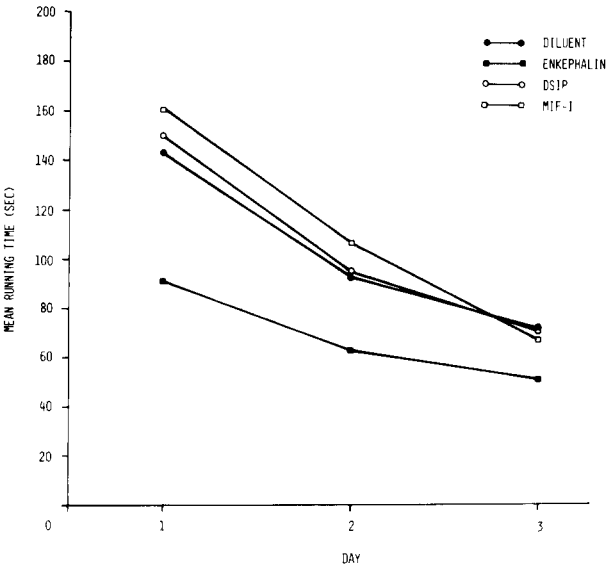

FIG. 1. Daily mean number of seconds for adult rats to run a 12-choice maze after SC injection of Met-enkephalin, MIF-I, DSIP, or diluent as infants.

TABLE 1

MEAN RUNNING TIMES (SEC) FOR ADULT RATS INJECTED NEONATALLY WITH 80 μg/kg SC MET-ENKEPHALIN, MIF-I, DSIP, OR DILUENT

Treatment	Rats	No.	Day 1	Day 2	Day 3
Met-enkephalin	Males	(12)	99.2	64.7	55.9
	Females	(13)	84.2	61.2	45.9
	Combined	(25)	91.4	62.9	50.7
DSIP	Males	(16)	163.4	104.6	72.0
	Females	(9)	125.8	77.4	57.7
	Combined	(25)	149.8	94.8	70.7
MIF-I	Males	(11)	137.5	96.7	64.5
	Females	(8)	193.8	120.0	81.8
	Combined	(19)	161.2	106.5	66.8
Diluent	Males	(15)	136.3	92.0	68.1
	Females	(9)	154.7	95.7	75.1
	Combined	(24)	143.2	93.4	71.7

RESULTS

Analysis of variance of all rats for running time revealed a significant main effect of peptide, $F(3,89)=3.64, p<0.02$, and day, $F(2,178)=58.54, p<0.01$. Duncan's Multiple Range Test showed that adult rats injected with Met-enkephalin during the first week of life ran the maze significantly faster than rats injected neonatally with diluent ($p<0.05$), DSIP ($p<0.05$), or MIF-I ($p<0.01$) on day 1. As was seen with Met-enkephalin injected into adults [9], the effect became less marked on succeeding days. This ceiling effect with improved learning is illustrated in Fig. 1.

As can be seen in Table 1, female rats injected with Met-enkephalin tended to run slightly faster than males on each of the three experimental days despite the fact that female controls injected with diluent tended to run slower than male controls. A similar trend was noted for DSIP, but in the groups receiving MIF-I, the males tended to run faster than the females.

Data obtained for errors were analyzed in the same manner as the data for running times. Analysis of variance revealed a significant main effect of peptide, $F(3,89)=3.78$, $p<0.02$ and day, $F(2,178)=44.97$, $p<0.01$. Duncan's Multiple Range Test showed that rats injected during the neonatal period with Met-enkephalin made significantly ($p<0.05$) fewer errors on both day 1 and 2 of testing than rats receiving diluent or MIF-I. The diminishing differences in number of errors among experimental groups as learning progressed, seen previously in the study using only adults [9], is illustrated in Fig. 2.

As was observed with running times, female rats injected neonatally with Met-enkephalin tended to make fewer errors than males on the first experimental day (Table 2). These females made significantly ($p<0.01$) fewer errors relative to the groups of female rats receiving diluent or MIF-I in which the opposite tendency was seen on that day.

No significant changes in the activities of tyrosine hydroxylase or choline acetyltransferase were found in the four parts of the rat brain examined in this study. There was a tendency, however, for tyrosine hydroxylase activity to be slightly higher in the cerebellar and striatal areas of brains from rats receiving neonatal injections of MIF-I and slightly lower in rats injected with DSIP.

RIA for DSIP-like material was performed on the brains of five adult rats of each sex injected during the first week of life with DSIP or diluent. The greatest difference appeared to occur in some brain parts of the female rats, where DSIP levels were higher in those injected neonatally with DSIP as compared with diluent. These areas included the thalamus (31.8 ± 4.3 vs. 23.2 ± 3.5 pg/mg), striatum (31.2 ± 3.7 vs. 22.4 ± 1.9 pg/mg), and the occipital cortex (28.5 ± 2.9 vs. 24.4 ± 2.3 pg/mg).

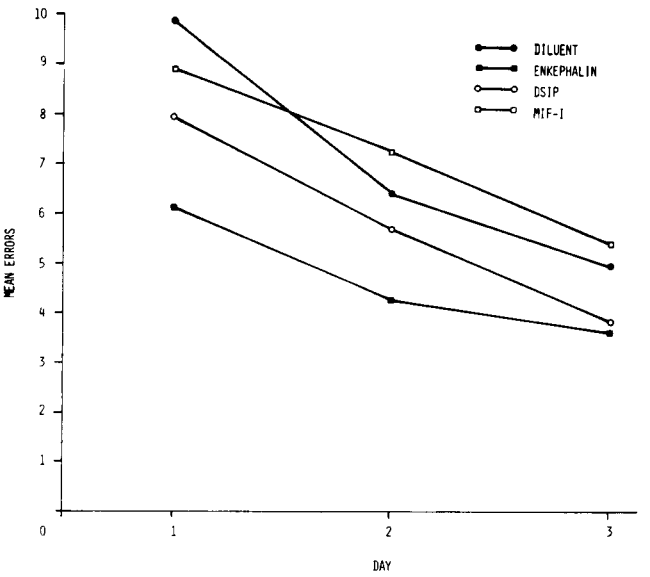

FIG. 2. Daily mean number of errors for adult rats to run a 12-choice maze after SC injection of Met-enkephalin, MIF-I, DSIP, or diluent as infants.

TABLE 2

MEAN NUMBER OF ERRORS FOR ADULT RATS INJECTED NEONATALLY WITH 80 μg/kg SC MET-ENKEPHALIN, MIF-I, DSIP, OR DILUENT

Treatment	Rats	No.	Day 1	Day 2	Day 3
Met-enkephalin	Males	(12)	6.50	3.79	2.92
	Females	(13)	5.77	4.69	4.27
	Combined	(25)	6.12	4.26	3.62
DSIP	Males	(16)	8.00	5.59	3.47
	Females	(9)	7.72	5.78	4.39
	Combined	(25)	7.90	5.66	3.80
MIF-I	Males	(11)	7.18	5.60	4.82
	Females	(8)	11.31	9.56	6.19
	Combined	(19)	8.92	7.26	5.39
Diluent	Males	(15)	8.07	5.59	4.17
	Females	(9)	12.39	7.72	6.28
	Combined	(24)	9.69	6.40	4.95

DISCUSSION

The results show that rats injected with Met-enkephalin during the first week of life ran a complex maze faster and with fewer errors as adults than rats injected with diluent, MIF-I, or DSIP. This indicates that in addition to its acute activational effects on maze behavior in the adult animal [9], Met-enkephalin may also have a role, when administered during the neonatal period, in altering the organization or development of processes involved in learning tasks such as the maze.

The female rats injected as infants with Met-enkephalin and DSIP appeared to learn the maze slightly faster than the male rats. This sex difference did not occur in the control rats injected with diluent in which an opposite tendency occurred. Males also tended to perform better than females when injected neonatally with MIF-I, a finding which parallels an earlier study [2] with alpha-MSH in which males performed better than females on a visual discrimination problem after neonatal injection of the peptide.

An increase in general arousal or activity does not seem to account for the findings in this study since the rats receiving Met-enkephalin made fewer errors, not more. This has been a consistent finding with those brain peptides which have been found to result in facilitated negotiation of the Warden maze [9, 11, 12]. In each study, peptides have been shown to increase acquisition of a complex appetitive task, indicating that their actions are not confined to avoidance tasks.

Previously, every peptide injected into newborn rats has resulted in long-term effects in adults [1, 2, 3, 5, 10, 11]. Although enkephalin also exerted chronic effects in this experiment, DSIP and MIF-I did not. However, instead of indicating that only some of the peptides known to exert CNS actions in adults are effective when injected neonatally, it is possible that a particular task or group of tasks are only affected by certain peptides. MIF-I previously has been shown to result in shorter latencies of adult rats running a maze, but doses ten times higher than in the present study were used under slightly different experimental conditions [11]. More recent preliminary studies with the 12-choice

maze under conditions identical to those used in the present experiment failed to find a reliable effect of either MIF-I or DSIP in adult male rats (unpublished observations).

Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of dopamine, norepinephrine, and epinephrine while choline acetyltransferase catalyzes the synthesis of acetylcholine. The activities of neither enzyme were significantly different in the four areas assayed in brains obtained from adult rats injected as infants with Met-enkephalin, DSIP, MIF-I, or diluent.

Since injected peptides persist in the blood for only a few minutes [8], it was not expected that brain levels of exogenous DSIP-like material would remain elevated for three months. Moreover, in order to detect, even transiently, any increased content of DSIP after peripheral administration, intracarotid injection of larger amounts are necessary [6]. It is possible, however, that early exposure to a peptide could affect later endogenous synthesis, storage, and release of that or other peptides or the sensitivity of target cells to the peptide. Our results raise the possibility that neonatal administration of DSIP may have slightly increased the levels of DSIP-like material in some brain parts of female rats killed three months later. This might represent increased endogenous material stimulated by the early exposure to DSIP.

The actions of Met-enkephalin in this study support our concept of a dissociation between behavioral and narcotic effects of the opiate peptides [8,9]. The persistence of these effects for several months suggests an action on the organization of the brain after neonatal administration.

ACKNOWLEDGEMENTS

Supported in part by the Medical Research Service of the Veterans Administration, NIH (NS 07664), and the American Parkinson Disease Foundation. The authors thank Michael D. Mauk for statistical analyses, Paul F. Castellanos and Cheryl Nissen for RIA, and Dr. James Zadina for review.

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