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BRIEF COMMUNICATION

Experience Affects Cortical But Not Subcortical Polyamines

P. A. FERCHMIN[1] AND VESNA A. ETEROVIĆ

Department of Biochemistry, School of Medicine
Universidad Central del Caribe, Cayey, Puerto Rico 00634

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FERCHMIN, P. A. AND V. A. ETEROVIĆ. *Experience affects cortical but not subcortical polyamines*. PHARMACOL BIOCHEM BEHAV **35**(1) 255–258, 1990.—The effect of brief periods of experience in an enriched environment (7 hours per day for 3 days), and of inhibition of polyamine synthesis was studied in four brain regions: occipital cortex, remaining cortex, subcortex and cerebellum plus medulla. Polyamine synthesis was inhibited by α-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase. DFMO caused a 30–50% decrease in putrescine content in all brain areas, irrespective of the environmental treatment. Spermidine was decreased by the inhibitor in subcortex and in cerebellum plus medulla, while spermine was increased in remaining cortex. The regional differences in inhibitor effect suggest that the regulation of polyamine metabolism varies among the four brain areas. Experience increased the weight and spermidine content of remaining cortex and decreased putrescine content of occipital cortex. Noncortical areas were not affected. The effects of experience on polyamine levels were somewhat increased by DFMO. Therefore, experience did not have a generalized effect on polyamine levels; rather, each polyamine responded in a specific manner. In addition, polyamine levels were affected only in those brain areas which are known from previous studies to respond to environmental stimulation with weight increase. These facts suggest that polyamines might have a role in the regulation of experience-induced plasticity.

Experience-induced plasticity Brain cortex Rat Putrescine Spermidine Spermine
α-Difluoromethylornithine

ENRICHED experience affects not only the behavior, but also the structure and ultrastructure (11) and biochemistry of brain cortex (13). The finding that less than 1 hour of exposure to a complex environment can produce significant changes in brain weight and RNA content indicates that this treatment is a powerful tool to induce brain plasticity (2). The biochemical processes underlying these experience-induced morphologic changes are not well understood. Increased RNA concentration accompanies the earliest stages of cortical weight increase and is probably a prerequisite for the weight increase to occur (2). Polyamines were shown to affect DNA, RNA and protein biosynthesis (7). They play an important role during brain development (12) and in the mature brain they might affect transmitter release (1), calcium metabolism (4) and vascular permeability (6) among others. In general, polyamines are crucial modulators of growth, proliferation and differentiation, as well as of several cellular mechanisms related to synaptic functions.

The effect of complex environment can be described as increased differentiation rather than proliferation of cortical neurons (11). On the other hand, inhibition of polyamine biosynthesis was reported to induce differentiation in cells of neural (5,8) and nonneural (9) origin. Therefore, it seems plausible that the inhibition of polyamine biosynthesis could enhance the effect of enriched experience by facilitating neuronal differentiation. Previously, we have inhibited polyamine synthesis in rats exposed to enriched or impoverished environments (3). We reported that such inhibition did not prevent the effect of experience in cerebral cortex; on the contrary it seemed to increase it slightly. Here we compare the data for cerebral cortex with those for other brain areas and show that experience modifies polyamine content only in brain cortex. The latter is the most plastic brain region in this experimental system as was observed repeatedly by many investigators using biochemical, microscopic and anatomical methods (13). Although experience-induced brain plasticity is not restricted to cortex, other brain areas show smaller plastic changes that are not detectable by weighing tissue sections.

The inhibitor of polyamine biosynthesis used in these experiments was α-difluoromethylornithine (DFMO). DFMO is an enzyme activated inhibitor of ornithine decarboxylase (ODC), the first and rate limiting enzyme of polyamine biosynthesis (10).

[1]Requests for reprints should be addressed to P. A. Ferchmin, Department of Biochemistry, School of Medicine, U.C.C.E.M., Cayey, P.O. Box 935, Puerto Rico 00634.

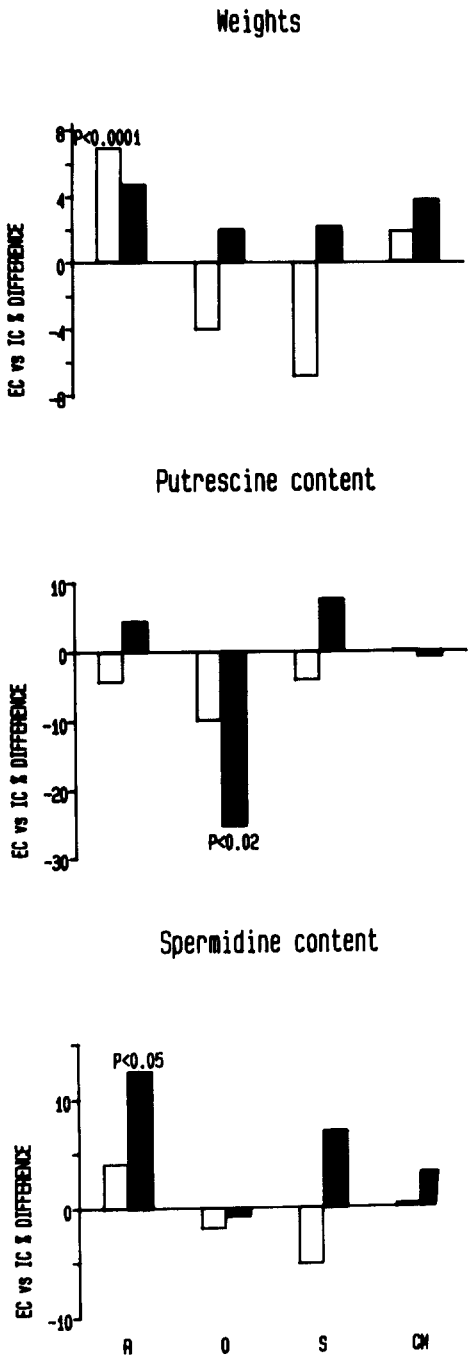

FIG. 1. Effect of environment on brain parameters in the presence or absence of DFMO: regional differences. EC vs. IC % difference of the means is shown for: weight of four brain sections, putrescine content (nmoles/section), and spermidine content of the same sections. White bars correspond to the % difference between saline-injected EC/IC pairs, black bars to DFMO-injected pairs. DFMO was injected twice a day (total daily dose 400 mg/kg), at the beginning and end of exposure to EC. The 4 brain sections were: remaining cortex, R; occipital cortex, O; subcortex, S; cerebellum plus medulla, CM. The data were analyzed and presented as explained in the Method section. The statistical significance of EC vs. IC differences are indicated where $p<0.05$. N was equal to 8 values per experimental conditions (32 values total) for all measurements in O, S, and CM. For the remaining cortex N was 7 for all measurements. The data for remaining cortex were reported previously (8) and are shown here to make possible the comparison with the other 3 areas. Standard errors of the bars from the upper left bar to the lower right one were: 0.9, 0.8, 3.6, 4.0, 1.5, 1.7, 1.3, 1.3; 3.4, 7.3, 5.2, 6.7, 3.1, 6.9, 4.4, 6.9; 3.9, 4.0, 5.9, 6.2, 2.3, 2.9, 2.8, and 3.0.

METHOD

The methods were those described in (3). Briefly, a total of 32 male Tryon S_1 rats were used. The animals were habituated to injections during 2 weeks before weaning; without habituation the injections impair the interaction with the complex environment (3). After weaning, at 30 ± 3 days of age, four male pups were taken from every litter and assigned at random to one of four experimental conditions: 1) EC-DFMO: rats kept in enriched condition (EC) 7 hours a day for three days, injected with DFMO 200 mg/kg before and after each daily exposure to EC (daily DFMO dose 400 mg/kg). 2) IC-DFMO: rats housed in impoverished condition (IC) for 3 days, injected with DFMO at approximately the same time as the EC-DFMO littermates. 3) EC-saline: same as EC-DFMO, but injected with saline. 4) IC-saline: same as IC-DFMO, but injected with saline. All rats were housed in individual cages $12 \times 20 \times 13$ cm. The littermates assigned to IC were housed in these cages for three days, being removed daily for weighing and injecting. The rats assigned to EC were removed from the individual cages and exposed to EC for 7 hours a day. The animals were killed the morning of the fourth day and the brains dissected under blind conditions following the method described in (2). Four sections were obtained from each brain: The cortex was subdivided into occipital cortex and remaining cortex (the latter included the hippocampus). The two other sections were subcortex and cerebellum plus medulla.

Polyamines were determined by benzoylation and quantification by HPLC following the procedure used previously (3).

The absolute values for brain weights and polyamine contents were normally distributed and homoscedastic. These data were analyzed by a two-factor (environment and drug) ANOVA for blocked data. The significance levels shown on figures were obtained from this analysis of the absolute values. Percent differences were calculated from the means (μ) of different experimental conditions, e.g.,

$$100[\mu(\text{EC-saline}) - \mu(\text{IC-saline})]/\mu(\text{IC-saline}) = \text{EC vs. IC \% difference for the saline group.}$$

Or:

$$100[\mu(\text{EC-DFMO}) - \mu(\text{EC-saline})]/\mu(\text{EC-saline}) = \text{DFMO vs. saline \% difference for the EC group.}$$

Standard errors (SE) of percent differences were calculated using the following formula, obtained by the method of error propagation:

$$SE = 100[MSE/16\ \mu_c^2 + \mu_e^2\ MSE/16\ \mu_c^4]^{1/2}$$

where MSE is the Error Mean Square from the ANOVA analysis, μ_c is the mean of the "control" group and μ_e is the mean of the "experimental" group. For example, in an EC vs. IC comparison, EC group would be considered "experimental" and IC group "control"; in a DFMO vs. Saline comparison, DFMO group would be "experimental" and the Saline group would be control.

RESULTS

Figure 1 shows the EC vs. IC differences in weights of brain

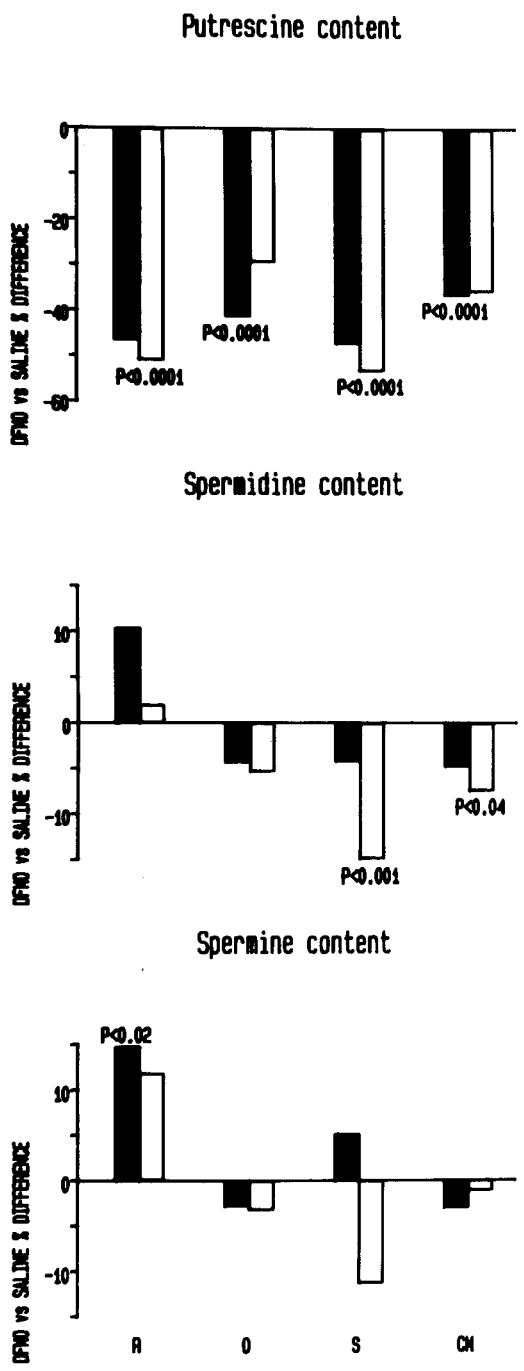

FIG. 2. Effect of DFMO on the content of putrescine, spermidine and spermine in EC (black bars) and IC (white bars) rats: regional differences. The 4 brain sections and the number of values per experimental condition were as indicated in Fig. 1. The significance of DFMO treatment is indicated for the corresponding brain areas. As indicated in legend to Fig. 1 the data for remaining cortex were reported earlier. SEM of the bars are presented as in Fig. 1: 2.9, 2.8, 4.5, 4.7, 2.6, 2.4, 3.7, 3.7; 3.8, 3.8, 5.9, 5.8, 2.5, 2.2, 2.7, 2.7; 5.1, 5.2, 5.2, 5.3, 3.5, 3.0, 3.5, and 3.8.

sections from rats injected with DFMO or saline. Remaining cortex was the only area that showed a significant weight increase in EC rats; occipital cortex did not show a weight increase. Occipital cortex is the most plastic part of the cortex in long term EC-IC experiments. However, short exposures to differential environments like the one used here (7 hours a day during 3 days) do not reliably alter this area (2). The weight of subcortical areas, cerebellum plus medulla and subcortex, are refractory to the effects of experience.

The effect of experience on polyamine content was restricted to the two cortical sections. Putrescine was decreased in occipital cortex, $F(1,21)=6.64$, $p<0.02$, while spermidine was increased in remaining cortex, $F(1,18)=4.43$, $p<0.05$, of EC rats (Fig. 1). Spermine content did not significantly change as a result of experience in EC (results not shown).

Figure 2 shows the effect of DFMO on polyamine content in brains of EC and IC rats. DFMO was effective in lowering putrescine levels in the four brain areas indicating that there was no major regional difference in DFMO inhibitory activity. The effect of DFMO on the content of spermidine and spermine presented regional differences. Spermidine was significantly decreased by DFMO in the subcortex, $F(1,21)=14.73$, $p<0.001$, and cerebellum plus medulla, $F(1,21)=4.96$, $p<0.04$; whereas spermine was increased in remaining cortex, $F(1,18)$, $p<0.02$.

DISCUSSION

The purpose of this experiment was to study the relatively early biochemical events that might be involved in triggering experience-induced plasticity. For that reason the environmental treatment was shorter than usual (30–90 days) at the expense of smaller EC vs. IC differences.

In this experiment the experience-dependent weight increase was restricted to the remaining cortex, which also showed an EC-dependent increase in spermidine content. Remaining cortex was the only area to show an increase in spermine by DFMO. This paradoxical increase in spermine was probably caused by decreased putrescine content since putrescine is an inhibitor of spermine synthase (10). However, a reduced spermine breakdown might also have contributed to the increase in spermine content. The finding that only remaining cortex showed this effect suggests that there are subtle differences among brain areas in the synthesis and recycling of polyamines.

Complex environment decreased significantly putrescine content in occipital cortex. The weight of this region was not increased in this experiment, probably due to the briefness of environmental treatment. Since occipital cortex weight always increases with longer exposures to EC (as demonstrated in innumerable previous studies), it is possible that the observed decrease in putrescine might be related to the later weight increase.

It is known that the weights of subcortex and cerebellum plus medulla are not changed by the EC treatment (2,13). In these regions polyamine levels were not affected by experience. In addition, the pattern of changes in polyamine levels caused by DFMO differed from that observed in the cortical regions.

In summary, the effect of DFMO on spermidine and spermine content was different in the four brain areas suggesting regional differences in polyamine metabolism. The effect of experience on polyamines was not generalized, but restricted to cortical areas whose weights can be increased by experience. Therefore, it is conceivable that polyamines are involved in experience-induced brain plasticity.

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