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Effects of Crowding on Dopamine-β-Hydroxylase Activity in Locus Coeruleus and Hypothalamus of Rats

Makoto Daiguji and Itaru Yamashita*

Abstract

The effects of crowding on dopamine-β-hydroxylase (DBH) activity were studied in locus coeruleus, anterior and posterior hypothalamus of rats and compared them with those of cold exposure in our previous report. In the case of 24 hour crowding, DBH activity changed the same as in the case of repeated cold exposure we reported previously. However in the case of 3 to 14 days of crowding, DBH activities did not differ from the control in any of the three brain regions. These results suggest that temporally different alteration could be brought on the regulation of brain DBH activities by different kinds of stress manipulation.

Introduction

Stressors such as imobilization, electrical shock ether, fromaldehyde, forced swimmwng or cold exposure have been employed so far in studies on the relations between stress and neurotransmitters in the brain. Any of these stressors can be regarded as those chiefly based upon physical factors. We are currently engaged in the study of the relations between stress and dopamine-β-hydroxylase (3,4-dihydroxyphenylethylamin, ascorbate: oxygen oxydoreductase, EC 1.14.17.1, DBH), a noradrenaline-synthesizing enzyme. Our previous report was the one on the effects of stress on the brain DBH activities in rats; cold exposure was employed as a physical stressor (Daiguji, *et al.*, 1982).

The purpose of the present study is to examine the effects of exposure of crowding, one of the socio-pychological stress, on DBH activities of the locus coeruleus and hypothalamus of rats and to compare the results with those of our previous report on the cold exposure.

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Materials and Methods

One male and two female healthy rats of Wistar strain were raised in a cage for a month and separated. Out of the pups born after a certain period female pups were excluded and male pups only were raised together with dams for 3 weeks and separated. The male pups were raised in a plastic cage with sawdust bedding and fed on MF (Oriental Yeast Co., Ltd.). All rats, the total number of which were 48, were housed three per standard laboratory cage (30 ×25 ×18cm) under artificial illumination (lights on at 6:30 and off at 18:30) before crowding experiments. The bedding was replaced once in two days and handling of rats was carried at the same time. Food and water were given ad libitum.

Crowding was given as follows: In the control group, rats were housed three per a standard laboratory cage just same as before experiments. In the crowding groups, six rats were housed into an experimental cage (21×14×13cm) made of the same plastic plate.

After starting the experiment of crowding, both the "crowding groups" and the "uncrowding groups" with bedding, handling, food and water everyday since inside of the cage used to become unclean.

Crowding was brought about by housing rats in the confined free floor space which was approximately 1/10 to 2/10 of the total area in the control condition. Three kinds of crowding manipulation were given; 5 min, 24 hour, 3 days and 14 days.

The "crowding groups" and the "uncrowding groups" were arranged to consist of the same number of rats from the same dam respectively (n=6). Furthermore, decapitation was scheduled to be performed on the 8th week after birth.

The rats were killed by decapitation without anesthesia at 14:00 hr ± 30 min in order to avoid the diurnal variation of plasma corticosterone concentration and DBH activities in the brain, which we reported previously (Daiguji *et al.*, 1978). After the brain was removed, the locus coeruleus (LC), anterior hypothalamus (aHt), and posterior hypothalamus (pHt) were dissected out by the method of Reis and Ross (1973), Glowinski and Iversen (1966), and Manshardt and Wurtman (1968). The dissected samples were homogenized in 0.1M phosphate buffer (pH 5.5) containing 0.1% of Triton X-100 and their supernatant fluids were stored at —70℃ for the DBH activity assay.

The trunk blood was collected in a heparinized tube and centrifuged (3000g×20 min). The plasma was separated and stored at-70℃. Plasma corticosterone was assayed by the method of Zenker and Bernstein(1958).

DBH activity was assayed by a two-wavelength microphotometric method of Kato *et al.* (1974) with 20mM of tyramine as substrate and was presented as n mol of octopamine formed per min per g wet tissue. N-ethylmaleimide (15mM), Cu^{2+} (1mM) and Sodium fumarate (10mM) were all optimal in our assay condition.

Results and Discussion

The mean plasma corticosterone concentration was markedly elevated in the 5 min crowding group (21.3±1.5 μg/dl) and it differed significantly from the control group (10.0±2.8 μg/dl, $p<0.01$, $n=6$), DBH activities of LC, aHt and pHt in the 5 min crowding group did not differ significantly from those of the control group (LC: 9.49±1.30 *vs* 7.81±1.08, aHt: 7.63±0.49 *vs* 6.59±0.45, and pHt: 4.12±0.66 *vs* 4.21±0.34, each value represents control group *vs* crowding group, mean± S.E. as nmol of octopamine formed per min per g wet tissue).

Rats exposed to a 24 hour crowding condition weighed the same as the control rats, and the plasma corticosterone concentration was not different from that of the control group. The DBH activity was significantly elevated at LC (24%) and decreased at pHt (39%) from the control group. At the aHt DBH activity was decreased (21%) but it was not statistically significant from the control group (Table 1).

Table 1. Effects of 24 hour crowding on DBH activity.

	control ($n=6$)	24hour crowding ($n=6$)
Locus coeruleus (LC)	9.65±0.72	11.93±0.73*
Anterior hypothalamus (aHt)	7.41±0.93	5.83±0.37
Posterior hypothalamus (pHt)	4.38±0.54	2.69±0.14**

Results are expressed as mean nmol of cotopamine formed per min per g wet tissue±S. E.
DBH activity showed an increase in locus coeruleus (LC) and a decrease in posterior hypothalamus (pHt).
* : $p<0.05$ *vs* control, ** : $p<0.02$ *vs* control.

In the case of 3, 7 and 14 days of crowding, the plasma corticosterone concentration was not different from that of the control group (control group ; 10.0±2.8, experimental group; 3days ; 12.7±3.6, 7 days; 12.7±3.1, 14days; 11.7±2.1, each value represents mean±S.E. as μg/dl), however the body weight of rats decreased markedly (control group ; 307±11g, experimental group; 3 days; 248±3g, 7 days; 243±6g, 14 days; 242±5g). Differences were statistically highly significant at $p<0.001$ from control values. $n=6$). Nevertheless the DBH activities of LC, aHt and pHt did not differ from the control group in any of these days (Table 2).

Table 2. Effects of 3,7 and 14 days crowding on DBH activity.

	contol ($n=6$)	crowding		
		3 days ($n=6$)	7 days ($n=6$)	14days ($n=6$)
Locus coeruleus (LC)	9.49±1.30	8.27±1.19	9.60±1.24	10.27±0.99
Anterior hypothalamus(aHt)	6.63±0.49	7.57±0.64	7.10±0.71	7.02±0.73
Posterior hypothalamus(pHt)	4.12±0.66	4.33±0.27	4.47±0.40	4.50±0.50

Results are expressed as mean nmol of octopamine formed per min per g wet tissue.
There was no significant change in any of the three regions.

Crowding is one of socio-emotional stress. Several studies showed over crowsing produced significantly higher moter activity (Kment, *et al.*, 1982), lower body weight in rats (Armario, *et al.*, 1984), several kinds of anxiety in human (Baxter; et al., 1970), and the elevation of plasma corticosterone concentration in rats (Barret and Stockham, 1963). In the present experiment, plasma corticosterone was markedly elevated during 5min crowding, indicating that our experimental condition did produce a stress response in these rats. The body weight of rats markedly decreased in 3 to 14 days of crwoding as Armario, *et al.* (1984) indicated. However there were no report on the effects of crowding on noradrenaline synthesising enzymes and this is the first report on it.

As our previous report showed (Daiguji, *et al.*, 1982), the DBH activities were increased at LC and decreased at aHt and pHt when cold exposure was repeated over 1-3 weeks. Similar alterations due to repeated stress have been reported for tyrosine hydroxylase (Zigmond, *et al.*, 1974) and the phenylethanolamine N-methyltransferase (PNMT) activities as well (Saavedra and Torda, 1980). When crowding was employed as a stressor, on the other hand, similar alterations in the DBH activities were obtained at the end of the first 24 hours and no alteration was observed after 3-14-days crowding.

The above results can be summarized as the following. Intracerebral DBH activity was increased at LC and decreased at aHt and pHt, which was the common aspect when the rat was exposed to some stress whether it was cold exposure, a mainly physical stressor, or it was crowding, a mainly psychological one. There were, however, some differences between the 2 stress in time course. That is, alterations in the DBH activities were continually observed over 1-3 weeks with cold exposure but were observed only at the end of the first 24 hours with crowding; for the latter, no alterations was obtained after that. This aspect suggests that temporally different alterations could be brought on the regulation of brain DBH activities by different types of stressors. Further investigations, however, would be needed to elucidate these points.

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