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Effect of Valproic Acid on Brain Serotonin Metabolism in Isolated and Grouped Rats

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KEMPF, E., G. MACK, C. SCHLEEF AND P. MANDEL. *Effect of valproic acid on brain serotonin metabolism in isolated and grouped rats.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 49–52, 1982.—The effect of the GABA transaminase inhibitor valproic acid (DPA) on serotonin (5HT) metabolism of different brain regions were studied in both grouped and isolated rats. One hour after DPA injection 5HT levels in the amygdala were increased in grouped and isolated rats. In the hypothalamus of grouped rats, changes in 5HT metabolism were also found. The alteration in 5HT metabolism in grouped rats was reversed 150 min after injection of DPA. At this same time, a large and significant increase in 5HT turnover was observed in all brain areas examined in isolated rats. It can be concluded that prolonged isolation induces a differential sensitivity to the effects of DPA leading to differences in 5HT metabolism: the drug effect being more intense in isolated rats.

Valproic acid Serotonin level and turnover Rat brain Isolation

IT is well known that prolonged isolation of mice [18,19] and rats [1] may induce aggressiveness and parallel decreases in serotonin metabolism (5HT) [21], glutamic acid decarboxylase activity [3], GABA levels [5,17] and of GABA binding [4] in the brain as well as changes in sensitivity to drugs as compared with grouped animals [9, 20, 22].

Valproic acid (n-dipropylacetic acid, DPA) is an effective agent in blocking mouse killing behaviour in the rat [11] and intraspecific aggression in mice [13,14]. DPA increases brain levels of GABA by inhibiting GABA transaminase [15], and a relationship between the increase in cerebral GABA concentration and the loss of the muricidal behaviour of spontaneous mouse killing rats has been reported [10]. That the potentiation of GABA mediated inhibition is involved in the blockade of muricidal behaviour is confirmed by the similar effects of GABA agonists and inhibitors of GABA uptake administered either locally in the olfactory bulb or by systemic injection [10].

Furthermore, changes in monoamine metabolism and particularly in serotonin metabolism have been observed after intraventricular administration of GABA [2], DPA [6, 7, 11] or other GABA transaminase inhibitors [12]. Moreover, in killer rats with lesions of serotonergic nuclei in the raphe, DPA no longer suppresses muricidal behaviour [11] showing that the integrity of the serotonergic system is necessary to obtain the blockade of muricidal behaviour by DPA.

The purpose of this paper was to investigate extensively the effects of DPA on brain 5HT level and metabolism in differentially housed rats.

METHOD

One hundred male Wistar rats, 25 to 30 days old were housed together in compartment of dimensions (50 h × 80 w × 180 l cm). After two months, half of them were isolated in individual Makrolon cages of dimensions (15 h × 21 w × 40 l cm).

The animals were maintained at a constant room temperature (22°C) on a 0700–1900 hr light, and 1900–0700 dark cycle and were given laboratory chow and water ad lib.

At the end of the 2 months isolation period the animals were tested for their isolation induced mouse killing behaviour. Only the nonaggressive isolated rats were further used for these experiments. Grouped controls rats were maintained together in a compartment for the same period.

The animals were killed by decapitation. The different brain areas were dissected, weighed, frozen and stored in liquid nitrogen. The tissues were homogenized in 10 vol of acid ethanol (ethanol-water-HCl 0.05 N 74:16:10) at 0° in a Potter Elvehjem homogenizer and then centrifuged at 10,000 g for 10 min. The amines were separated on columns of Amberlite CG 50. Serotonin was separated by reverse-phase high performance liquid chromatography on a μ Bondapack-phenyl column (Water Assoc.), and estimated by electrochemical detection using a CP-oil type carbon paste electrode with a LC_4 thin-layer electrochemical detector as previously described [8].

Turnover parameters were determined by blocking the degradation of serotonin by pargyline (75 mg/kg, 45 min before sacrifice). Regression lines were calculated from the least squares fit.

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TABLE 1

EFFECTS OF DPA (200 mg/kg IP) UPON SEROTONIN METABOLISM IN DIFFERENT BRAIN AREAS OF GROUPED RATS ONE HOUR AFTER ADMINISTRATION

	Control		DPA	
Brain areas	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)
Amygdala	769 ± 72 (8)	792 ± 46 (16)	926 ± 71 (8)‡	639 ± 42 (16)
Lateral hypothalamus	871 ± 189 (8)	1052 ± 132 (15)	956 ± 188 (8)	1305 ± 128 (15)*
Medial hypothalamus	1024 ± 157 (7)	1320 ± 102 (15)	1053 ± 210 (8)	1689 ± 147 (15)†
Hippocampus	647 ± 65 (8)	525 ± 62 (15)	627 ± 55 (7)	664 ± 87 (15)
Pons medulla	1052 ± 152 (7)	1076 ± 133 (13)	1218 ± 219 (8)	923 ± 132 (15)

Numbers in parentheses indicate number of animals.
Significant differences based on Student's t-test: *$p<0.02$ vs controls, †$p<0.01$ vs controls, ‡$p<0.001$ vs controls.

TABLE 2

EFFECTS OF DPA (200 mg/kg IP) UPON SEROTONIN METABOLISM IN DIFFERENT BRAIN AREAS OF ISOLATED RATS ONE HOUR AFTER ADMINISTRATION

	Control		DPA	
Brain areas	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)
Amygdala	785 ± 68 (8)	806 ± 64 (14)	967 ± 81 (8)*	700 ± 66 (16)
Lateral hypothalamus	903 ± 108 (6)	994 ± 157 (13)	942 ± 111 (7)	1143 ± 135 (15)
Medial hypothalamus	992 ± 311 (8)	1438 ± 184 (16)	901 ± 209 (8)	1764 ± 222 (16)
Hippocampus	679 ± 62 (7)	579 ± 106 (14)	743 ± 89 (8)	681 ± 99 (15)
Pons medulla	1137 ± 201 (8)	1010 ± 133 (16)	1223 ± 206 (8)	1132 ± 123 (16)

Numbers in parentheses indicate number of animals.
Significant differences based on Student's t-test: *$p<0.001$ vs controls.

Brain 5HT levels and turnover were measured at different times after 200 mg/kg IP injection of DPA.

RESULTS AND DISCUSSION

There are no changes in 5HT metabolism in different brain areas of nonaggressive isolated rats as compared with grouped rats (Tables 1 and 2).

The effects of DPA in grouped rats upon 5HT metabolism in different brain areas known to be implicated in aggressive behaviour: amygdala, hypothalamus, pons medulla (raphe area) and in hippocampus are shown in Table 1. Sixty min after injection of DPA the GABA levels have reached their maximal value [16]. The 5HT level in the amygdala also was significantly higher after DPA injection compared to the control rats. DPA has no effect on 5HT level in all other regions examined. 5HT turnover was significantly increased only in lateral and median hypothalamus.

The results in Table 2 show that in isolated rats, DPA also produced a significant increase in amygdala 5HT levels one hour after injection but at this time there is no increase in 5HT turnover.

One hundred fifty min after injection of DPA in grouped rats, the observed changes had reverted to control levels (Table 3). In contrast, in isolated rats all brain regions examined, showed a significant increase in the turnover of 5HT after DPA administration. This increase ranged from +39% value for pons medulla to +63% for amygdala (Table 4).

The present data indicate a more prolonged period of elevation of 5HT turnover in the brains of isolated DPA-treated rats as compared with grouped rats. The drug effect differs not only in duration but also in intensity, being greater in isolated rats. Moreover there are marked regional differences in the effects of DPA on 5HT turnover.

Valzelli *et al.* [20] reported a more prolonged action of caffeine on 5HT and 5HIAA levels in whole brain of isolated aggressive mice as compared with grouped nonaggressive mice.

Following injection of 400–600 mg/kg DPA increases in whole brain 5HIAA levels have been observed in DBA/2 mice [6]. An increased tryptophan and 5HIAA level has been demonstrated in seven regional rat brain areas 30 min after 400 mg/kg DPA injection [7]. A nonstatistically significant increase in midbrain, striatum, hypothalamus and medulla 5HT turnover was also found to occur by the same authors. However, these authors assume that changes in small fractional pool of 5HT may not always be reflected by the measurement of 5HT accumulation after MAO inhibition.

TABLE 3

EFFECTS OF DPA (200 mg/kg IP) UPON SEROTONIN METABOLISM IN DIFFERENT BRAIN AREAS OF GROUPED RATS 150 MINUTES AFTER ADMINISTRATION

	Control		DPA	
Brain areas	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)
Amygdala	817 ± 69 (8)	770 ± 85 (16)	803 ± 150 (8)	691 ± 91 (16)
Lateral hypothalamus	930 ± 105 (8)	959 ± 94 (16)	1084 ± 110 (7)	1023 ± 122 (15)
Medial hypothalamus	909 ± 97 (7)	1166 ± 80 (15)	905 ± 142 (8)	1353 ± 109 (16)
Hippocampus	549 ± 84 (8)	561 ± 61 (16)	560 ± 89 (8)	512 ± 46 (16)
Pons medulla	1126 ± 64 (8)	934 ± 99 (16)	1214 ± 130 (8)	1091 ± 97 (16)

Numbers in parentheses indicate number of animals.

TABLE 4

EFFECTS OF DPA (200 mg/kg IP) UPON SEROTONIN METABOLISM IN DIFFERENT BRAIN AREAS OF ISOLATED RATS 150 MINUTES AFTER ADMINISTRATION

	Control		DPA	
Brain areas	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)	Steady state level (ng/g w.w. ± S.D.)	Turnover rate (ng/g/hr ± S.D.)
Amygdala	763 ± 90 (8)	687 ± 59 (16)	785 ± 100 (8)	946 ± 85 (16)†
Lateral hypothalamus	857 ± 85 (8)	1185 ± 94 (15)	942 ± 152 (8)	1984 ± 190 (16)†
Medial hypothalamus	908 ± 117 (8)	1459 ± 99 (16)	866 ± 58 (7)	2162 ± 161 (15)†
Hippocampus	561 ± 90 (8)	499 ± 58 (16)	477 ± 93 (8)	1038 ± 115 (16)*
Pons medulla	1247 ± 126 (8)	985 ± 65 (16)	1210 ± 86 (8)	1472 ± 140 (15)†

Numbers in parentheses indicate number of animals.
Significant differences based on Student's-t-test: *$p<0.02$ vs controls, †$p<0.01$ vs controls.

The present study indicates that there is no effect of isolation on the 5HT metabolism in the different brain areas of nonaggressive isolated rats. These results are consistent with the data of Valzelli [21], that showed only changes in 5HT metabolism in the mice which became aggressive after isolation. However, after DPA injection there are striking differences in 5HT metabolism of several brain areas of isolated rats, while in only two areas less pronounced changes occur in nonisolated rats. In view of the direct effect of DPA on GABA levels in the same areas [15], it seems likely that changes in 5HT neurotransmission which are not directly expressed after isolation, can be expressed when a potentiation of GABAergic system is produced.

In conclusion, DPA produces a slight but significant increase of 5HT levels in the amygdala of grouped as well as of isolated rats. Striking changes in 5HT turnover after DPA injection occur in addition in several other brain areas but only in isolated rats. In view of the increase of GABA in these same areas after DPA injection, it is suggested that isolation has sensitized serotonin turnover to a GABAergic neurotransmission.

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