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Effects of n-di-Propylacetate on Aggressive Behavior and Brain GABA Level in Isolated Mice

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SIMLER, S., S. PUGLISI-ALLEGRA AND P. MANDEL. *Effects of n-di-propylacetate on aggressive behavior and brain GABA level in isolated mice.* PHARMACOL BIOCHEM BEHAV 18(5)717–720, 1983.—n-di-Propylacetate (nDPA, valproate) a GABA-T inhibitor, injected IP at the dose of 300 mg/kg antagonized agonistic behavior of isolated DBA/2 mice in a time-dependent fashion in parallel to an increase of GABA levels in olfactory bulb, striatum, posterior colliculus and septum. After 75 min, aggressive responses were higher than those after 15 to 45 min and significantly lower in comparison with those of saline injected mice. After 120 min aggressive behavior was not different from that of control mice. The concentration of GABA in the striatum and olfactory bulb returned to control value 75 and 120 min after drug administration, respectively. After 120 min GABA levels in posterior colliculus and septum were lower than those after 15 to 75 min, although significantly higher in comparison with those of saline injected mice. The results are discussed in terms of the possible involvement of olfactory bulb and striatum in GABA-mediated control of isolation-induced aggressive behavior in mice.

GABA n-di-Propylacetate Isolation Aggressive behavior Mice

IN RECENT years some evidence was given indicating that the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) may play an important role in the control of different kinds of aggressive behavior such as mouse killing by rats, isolation and shock-induced aggressive behavior in mice and female aggression against lactating intruders [5, 7–13]. Concerning isolation-induced agonistic behavior, DeFeudis *et al.* [3] reported that the binding capacity of a heavy synaptosomal fraction was lower in the brains of isolated mice than in those of grouped ones. A decrease of glutamic acid decarboxylase activity in the brains of isolated mice has also been observed [1]. The tendency of exhibit aggressive responses was shown to be inversely related to GABA concentration in certain brain regions of isolated outbred strains of mice [4].

Recently, it has been reported that after a prolonged period of social isolation, mice of the C57B1/6 and DBA/2 strains are characterized by a decrease of GABA levels in olfactory bulbs, posterior colliculus, septum and striatum, DBA/2 mice showing lower levels of neurotransmitter in olfactory bulbs and striatum. Moreover, only DBA/2 mice showed a clear increase of aggressive responses [19]. In light of previous evidence [5, 7–13] indicating an inverse relationship between brain GABA levels and the display of aggressive behavior, these findings may suggest that the aggressive responses exhibited by isolated DBA/2 mice were related to levels of the inhibitory neurotransmitter in the above mentioned brain areas. Among these brain areas, olfactory bulb, striatum and septum are known to play an important role in the control of aggressive behavior [20,22] whereas the role of posterior colliculus in the expression of aggressive behavior is not yet known. The purpose of this study was to investigate the effects of n-di-propylacetate (nDPA) (a GABA-transaminase inhibitor) [17] on aggressive behavior and GABA levels in four brain structures of isolated aggressive mice: olfactory bulbs, posterior colliculus, striatum and septum.

In our experiments we used mice of the DBA/2 strain which display intense aggressive responses after social isolation in contrast to other strains such as C57B1/6 mice which do not exhibit aggressive responses after a prolonged period of isolation [16,19].

METHOD

Animals

Male mice (Charles River, France) of the DBA/2 strain (n=329) aged 11–12 weeks and weighing 21–24 g at the beginning of the experiments were individually housed (isolated) in opaque breeding cages (27×21×13.5 cm) for 8 weeks. These mice were given food (Standard Purina Chow DIET) and water ad lib in a 12/12 hr light/dark cycle. The experiments were carried out during the light period.

Behavioral Assessment

Aggressive responses and motor activity were assessed by means of a method previously described [13]. The latency

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of the first fighting episode, the number and the total time of fighting between two mice were automatically recorded for a 10 min session. The motor activity of two interacting mice was measured concomitantly to aggressive behavior. A total of eight pairs of naive mice for each experimental group were tested.

Brain GABA Levels

Naive mice (7 to 16 for each brain areas and experimental group) were sacrified by focussed microwave irradiation of the head (Litton, LMN, 70/50, 2 kW, 2.45 gHz, 3 sec) and decapitated. Brains were removed, dissected into anatomical areas, stored in liquid nitrogen and liophylised all as previously described [18]. GABA extraction and determination were performed on the brain areas according to the dansylation method of Seiler and Wiechmann [15]. The stable fluorescent dansyl derivative can be separated from other compounds present by one dimensional chromatography on silicagel G layers/two runs in diethyloxide: cyclohexane (6:4, v/v) and can be quantitatively determined by direct scanning of the thin layer plates in the range of 0.1 to 5 nanomoles/spot on an Aminco-Bowman Spectro-fluorimeter.

Drugs

nDPA (300 mg/kg) (LABAZ, Ambares) was dissolved in 0.9% NaCl and injected intraperitoneally at the volume of 10 ml/kg. NaCl (10 ml/kg) was used for control injections. Behavioral tests and brain removal were made 15, 30, 45, 75 and 120 min after the injections, and the results were compared with those of saline injected mice.

Statistics

The results were statistically evaluated by single-factor analysis of variance (ANOVA, Independent). Student's *t*-test were carried out in order to compare individual treatments.

RESULTS

The results showed that nDPA (300 mg/kg) decreased aggressive responses while increasing GABA levels in the four brain areas in a time dependent fashion (Figs. 1 and 2). The effects of nDPA on aggressive behavior and brain GABA levels were evident 15 min after the injection. The concentration of GABA in the striatum and olfactory bulb returned to control value 75 and 120 min after drug administration, respectively. After 120 min GABA levels in posterior colliculus and septum were lower than those after 15 to 75 min, although significantly higher in comparison with those of saline injected mice. After 75 min aggressive responses showed a significant increase in comparison with those exhibited after 45 min (latency: $p<0.01$; fighting episodes: $p<0.001$; fighting time: $p<0.01$) while except for latency, they were significantly different from those of control mice (Fig. 1). After 120 min aggressive responses of nDPA injected mice did not differ with statistical significance from those of saline injected mice (Fig. 1). For each interval from injection, activity assessment did not show any sedative effect of nDPA [11,12].

In additional experiments we observed that grouped DBA/2 mice of the same age as isolated mice used in the present study, did not show aggressive responses in our experimental conditions, as previously reported [16,19]. Moreover, GABA levels in septum and posterior colliculus

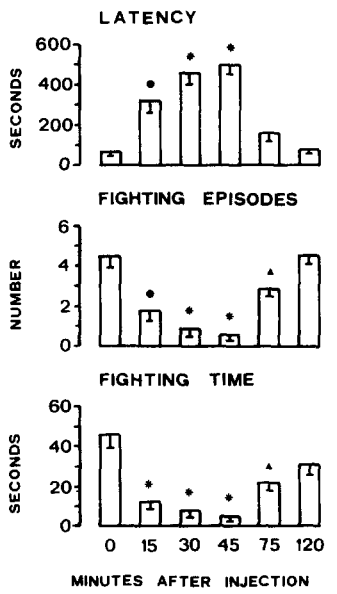

FIG. 1. Time dependent effects of nDPA (300 mg/kg IP) on aggressive behavior of isolated DBA/2 mice. Aggressive responses were expressed by the latency to the first fighting episode (Latency), the number (Number) and the total time (Time) of fighting episodes during a 10 min testing session. Mice were tested 15, 30, 45, 75 and 120 min after the injection and aggressive scores were compared with those of saline injected mice (0). Those couples of mice that failed to fight during a ten minute experimental sessions were assigned a maximum latency score of 600 sec. For the three parameters overall ANOVA showed a significant treatment main effect; Latency, $F(5.42)=11.37$, $p<0.001$; Number, $F(5.42)=13.23$, $p<0.001$; Time, $F(5,42)=10.17$, $p<0.001$. Individual between group comparisons showed significant differences between mice injected with saline and those injected with nDPA at different intervals from the injection: Latency=15 min ($p<0.01$), 30 min ($p<0.001$), 45 min ($p<0.001$); Number=15 min ($p<0.01$), 30 min ($p<0.001$), 45 min ($p<0.001$), 75 min ($p<0.05$); Time=15 min ($p<0.001$), 30 min ($p<0.001$), 45 min ($p<0.001$), 75 min ($p<0.05$). ▲=$p<0.05$; ●=$p<0.01$; *=$p<0.001$ in comparison with saline (0).

of grouped mice were not significantly different from those of isolated mice injected with nDPA (300 mg/kg) 120 min before testing. GABA levels in striatum and olfactory bulb of grouped mice were significantly higher than those of isolated mice injected with nDPA 75 and 120 min after injection, respectively.

DISCUSSION

The present results show that nDPA (valproate) administration resulted in a clearcut decrease of aggressive responses in isolated mice without affecting motor activity [11,12]. These effects of nDPA were evident 15 min after systemic injection and lasted for more than 45 min. Aggressive responses increased after 75 min and reached the initial levels 120 min after the injection.

Moreover, nDPA increased GABA levels in olfactory bulb, striatum, septum and posterior colliculus in a time-dependent fashion but with different temporal patterns depending on the brain area. In fact, GABA levels in septum and posterior colliculus were significantly higher than those of control mice 120 min after injection, while in striatum and

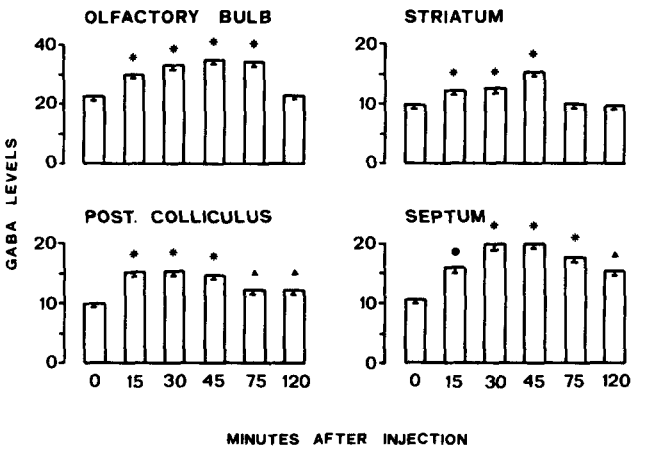

FIG. 2. Time dependent increase GABA levels in four brain areas following nDPA (300 mg/kg, IP) injection. The results are expressed in μmole/g dry weight ±S.C. ($7<n<16$). For the four brain areas overall ANOVA showed a significant treatment mean effect: olfactory bulb, $F(5.51)=22.18$, $p<0.001$; striatum, $F(5.42)=20.51$, $p<0.001$; posterior colliculus, $F(5.56)=8.11$, $p<0.001$; septum, $F(5.60)=12.16$, $p<0.001$. Individual between-group comparisons showed significant differences between mice injected with saline and those injected with nDPA at different intervals from the injection: Olfactory bulb=15 min ($p<0.001$), 30 min ($p<0.001$), 45 min ($p<0.001$), 75 min ($p<0.001$); Striatum=15 min ($p<0.001$), 30 min ($p<0.001$), 45 min ($p<0.001$); Posterior colliculus=15 min ($p<0.001$), 30 min ($p<0.001$), 45 min ($p<0.001$), 75 min ($p<0.05$), 120 min ($p<0.05$); septum=15 min ($p<0.01$), 30 min ($p<0.001$), 45 min ($p<0.001$), 75 min ($p<0.001$), 120 min ($p<0.05$). ▲=$p<0.05$; ●=$p<0.01$; *=$p<0.001$ in comparison with saline (0).

olfactory bulb they reached the initial levels 75 and 120 min after injection, respectively.

It must be pointed out that 75 min after drug administration aggressive responses were significantly higher than those exhibited by mice tested 45 min after injection; moreover after 120 min they were higher than those at 75 min but not significantly different from the initial levels. This gradual increase of aggressive responses parallels changes in GABA levels in striatum and olfactory bulb.

It is worth noting that as long as GABA levels in striatum and olfactory bulb were as high as those of grouped mice which do not show aggressive behavior in our experimental conditions, aggressive responses of isolated mice were very low.

These results are consistent with some studies which have recently pointed out an inverse relationship between GABA levels in the brain and aggressive responses in different kinds of aggressive behavior such as mouse killing behavior by rats [7, 9, 10], shock-induced [13] and isolation-induced [2, 4, 11] aggressive behavior in mice. In particular, Da Vanzo and Sydow [2] reported that aminooxyacetic acid (AOAA) and gamma-acetylenic GABA (GAG) produce a suppression of isolation-induced agonistic behavior in mice and an increase of brain GABA levels. These authors also observed that after injection of AOAA and GAG, the behavior of isolated mice was reversed at the time when brain GABA was significantly elevated. Thus our results may appear to be non-consistent with those of DaVanzo and Sydow [2]. It must be point out, however, that this apparent discrepancy may depend on the fact that DaVanzo and Sydow [2] in their study examined the whole brain, thus it can not be ruled out that their results mask some difference in GABA levels occurring in different brain areas. Moreover AOAA and GAG produce an increase in GABA mainly in cytosol while DPA produces an increase of GABA in nerve endings [6,14]. It is worth noting that we observed that GABA levels in nerve endings [6,14]. It is worth noting that we observed that GABA levels in septum and posterior colliculus were significantly elevated at the moment when aggressive responses reached the initial levels, while in the olfactory bulbs and striatum they were as low as before injection of nDPA.

The maintenance of GABA levels higher than those of control mice in the septum and posterior colliculus 75 and 120 min after drug administration might be explained either by a difference in diffusion or maintenance in situ of nDPA or by the relationship between GAD and GABA-T which play a fundamental role in GABA increase when GABA-T is inhibited.

Simler *et al.* [19] have recently reported that after 8 weeks of isolation, DBA/2 mice and not the C57B1/6 ones showed a clear increase of aggressive responses, thus confirming previous results [16]. Moreover both DBA/2 and C57B1/6 mice showed a decrease of GABA levels in olfactory bulb, striatum, septum and posterior colliculus. DBA/2 when compared to C57B1/6 were characterized by significantly lower levels of GABA in olfactory bulb and striatum. On the basis of these results the hypothesis arose as to whether the aggressive responses exhibited by isolated DBA/2 mice and not by isolated C57B1/6 mice might be related to lower levels of the inhibitory neurotransmitter in the olfactory bulb and striatum. It must be pointed out that in preliminary experiments we observed that nDPA produces an increase of brain GABA levels also in C57B1/6 mice. However it is not possible to relate this finding to aggression since isolation does not result in an increase of agonistic behavior in this strain.

Our present results seem to indicate that GABA levels in olfactory bulb and striatum may play an important role in the control of isolation-induced aggressive behavior in mice. In particular, nDPA resulted in a decrease of aggressive responses and in an increase of GABA levels in olfactory bulb, striatum, septum and posterior colliculus. This effect on aggressive behavior gradually disappeared while at the same time GABA levels reached their initial levels, firstly in striatum and then in olfactory bulb. On the other hand, GABA levels in septum and posterior colliculus were still significantly high at the time when aggressive behavior reached initial levels, thus indicating a possible lack of involvement of GABA functions in these brain areas in the control of isolation-induced aggressive behavior.

It must be taken into account, however, that the observed effects of nDPA on aggressive behavior may also depend on its action on other brain areas as well as on other neural networks involving other neurotransmitters. In fact a decrease of brain serotonin level or turnover rate in mice and rats after prolonged socio-environmental deprivation [22] and in killer rats [9] has been described. Moreover it has been shown that alterations of neurotransmitter systems other than GABAergic (e.g., the increase of choline acetyltransferase in the amygdala, the increases of dopamine turnover in the olfactory tubercule, and the decrease of serotonin turnover in pons-medulla) are also compensated by valproate injections in parallel with its inhibition of the aggression behavior pattern [9].

Further investigations are necessary in order to clarify the

interactions between GABA and other neurotransmitters in various brain regions and the role that these neurotransmitters play in the control of the behavioral effects of social isolation.

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