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Studies on the Involvement of GABA in the Aggression Directed by Groups of Intact or Gonadectomized Male and Female Mice Towards Lactating Intruders

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HAUG, M., S. SIMLER, L. KIM AND P. MANDEL. *Studies on the involvement of GABA in the aggression directed by groups of intact or gonadectomized male and female mice towards lactating intruders.* PHARMAC. BIOCHEM. BEHAV. 12(2) 189–193, 1980.—The aims of the present studies were (a) to determine the effects of pharmacological elevation of GABA by treatment with DPA (competitive inhibitor of GABA transaminase) on a form of aggression displayed by grouped female mice towards lactating and non-lactating intruders; (b) to estimate GABA levels in six different brain areas of intact and gonadectomized male and female mice. The results revealed that DPA treatment considerably reduced this form of aggression. Increased GABA levels, modulated by the hormonal state of the animals, were observed in most brain areas studied.

Aggression Brain Gonadectomy GABA Lactation

THE term "aggression" includes several distinct kinds of behavioral patterns, including predatory, inter-male, fear-induced, irritable, territorial, maternal and instrumental responses [22]. Recently, a type of aggressive behavior has been described in grouped female mice which does not appear to fit easily into any of the above categories. This occurred when resident animals were submitted to strange female conspecifics, particularly when the latter was lactating [7]. Furthermore, it was shown that attack behavior by female mice was released by chemical signals [8,9] and was dependent on strain [11], sex [12], and gonadal hormones [9,2]. For example in contrast to females, adult male residents do not increase their attacks on lactating intruders. After castration, however males show a dramatic increase in their attacks on lactating females which ceases after their hormones are replaced using either testosterone or oestradiol benzoate [12]. In contrast, ovariectomy had little influence on the aggressive responses of resident females to lactating intruders, in accord with previous reports [6,17].

These behavioral consequences of hormones are probably mediated by one or more of the following type of factors proposed by Beach [1]: (a) alteration of effector mechanisms used in the execution of specific behavioral patterns; (b) modification of sensory and perceptual mechanisms in such a way as to influence the reception or interpretation of environmental stimuli; (c) alteration of central neural mechanisms responsible for integration of incoming information or for organization of overt response patterns.

Recently, attention has been directed to the possibility of modifying interactions of hormones and central neurotransmitters to change aggressive behavior. Thus, sex-hormone and species-dependent chronological changes in the cerebral contents of serotonin [17], acetylcholine [4] and other biogenic amines [14] have been demonstrated. Gamma-Aminobutyric acid (GABA) may have an inhibitory action on muricidal behaviour in rats [18, 19, 20, 21]. Some studies in male mice have indicated that his putative neurotransmitter can be influenced by some environmental, social and hormonal factors that also influence aggression [3]. The present studies were undertaken to determine: (a) the effects of elevation of GABA level by a pharmacological agent on aggressive behaviour directed by groups of female mice towards lactating and non-lactating intruders; (b) to assess whether intact and gonadectomized male and female mice (known to differ on the basis of this form of attack) showed parallel variations in GABA content in six different brain areas following n-dipropylacetate (nDPA) treatment.

EXPERIMENT 1

EFFECT OF DPA ON AGGRESSION OF FEMALE MICE TOWARD LACTATING AND NON LACTATING INTRUDERS: A DOSE-RESPONSE STUDY

(nDPA) was used in this experiment because it increases brain GABA content [5], a factor which may be involved in its inhibition of muricidal behavior in rats [18].

TABLE 1

EFFECT OF nDPA ON FEMALE AGGRESSION TOWARD LACTATING AND NON-LACTATING STRANGERS

Treatment of female residents	Physiological state of intruder mice	Aggression test parameters: Mean number of attacks ± SE	Aggression test parameters: Mean latency of the first bite ± SE
NaCl control	Lactating	27.5 ± 3.42	63.1 ± 29.14
	Non-lactating	12.9 ± 3.80	534.0 ± 108.77
200mg/kg nDPA	Lactating	1.5 ± 1.00	798.3 ± 67.85
	Non-lactating	0	>900
300 mg/kg nDPA	Lactating	0	>900
	Non-lactating	0	>900

METHOD

Animals

One hundred and eighty female Swiss Albino mice were employed as "residents" in this experiment. They were derived from seventy litters each of which was reduced to eight animals which were randomly maintained within a closed colony. After weaning (30 days), all females were kept as groups of three in transparent Makrolon cages (33×12×18 cm). Mice were maintained on a sawdust substrate; food and water were available ad lib.

Thirty isolated lactating females (with eight pups) and 30 non-lactating females (housed in groups of five, except for the period of testing) served as "intruders". Their ages were comparable to those of residents.

One month after forming the residential groups, the animals were randomly allocated to one of the following treatment categories before being subjected to strange intruders: (a) twenty groups received an IP injection of DPA isotonic NaCl at the dose of 300 mg/kg; (b) twenty groups received DPA (200 mg/kg, IP); (c) the remaining groups (controls) were injected with isotonic saline.

Aggression Testing

Fifteen-minute aggression tests were begun, 10 min after injections. Half of the animals (10 groups of three) in each category of resident mice were subjected to lactating female (since two weeks) intruders, whereas the others encountered non-lactating intruders. Each intruder was randomly selected and used only once with a resident group. Sawdust bedding in the cages of the experimental animals was replaced with fresh material on the day preceding testing. Aggression tests included: (a) latency to first bite; (b) total attacks directed toward the intruders. Encounters were staged between 13:00 and 19:00 hr in the light portion of the animal's light-dark cycle. They were performed in a soundproof enclosure with isotropic lighting [7].

RESULTS

The results are summarized in Table 1. Administration of 300 mg/kg DPA totally inhibited the resident's aggressive responses. Animals treated with the lower dose of DPA (200 mg/kg) showed a very similar alteration of their aggressive behavior; no aggression was displayed toward non-lactating intruders, and the responses toward lactating females were significantly decreased when compared with the results obtained with both control groups (respectively: $t(18)$ (attack)=10.03 ($p<0.001$) and 2.78 ($p<0.02$); $t(18)$ (latency)=9.96 ($p<0.001$) and 2.45 ($p<0.05$).

EXPERIMENT 2

RELATION BETWEEN BRAIN GABA CONCENTRATIONS AND AGGRESSION DISPLAYED BY INTACT AND GONADECTOMIZED MALE AND FEMALE MICE TOWARD LACTATING INTRUDERS

The considerably reduced aggression observed among DPA-treated animals suggested (c.f. [24,25]) that the increased brain GABA level was correlated with the depressed fighting behavior. Consequently, the effects of pretreatment with DPA on the GABA contents of six brain areas from different resident mice previously subjected to lactating intruders were measured. These areas were: hypothalamus, olfactory bulbs, amygdala, anterior colliculus, posterior colliculus, and frontal cortex.

Since differences in aggressive behavior seemed dependent on the sex and hormonal state of mice, the following categories of resident animals were used: (a) intact and ovariectomized females; (b) intact and castrated males.

METHOD

Animals

Swiss albino animals were used throughout this study. Two hundred and forty mice were allocated to groups of three littermates (forty groups of each sex) at 30 days of age, and kept in cages measuring 33×12×18 cm. At seven weeks of age, half of the mice were castrated or ovariectomized under Nembutal (5%) anesthesia, and the remaining grouped males and females were submitted to sham-operations. After a 30-day recovery period, half of the mice of each category were randomly assigned to categories receiving 200 mg/kg DPA injections or 0.9% saline injections. The lower dose of DPA was selected for this experiment because higher doses may produce sedation. This study was limited to aggression toward lactating females, because this type of intruder most efficiently stimulates attacks from residents [7].

Aggression Testing

Testing was identical to that used in Experiment 1. Lactating strangers were randomly introduced into the resident's

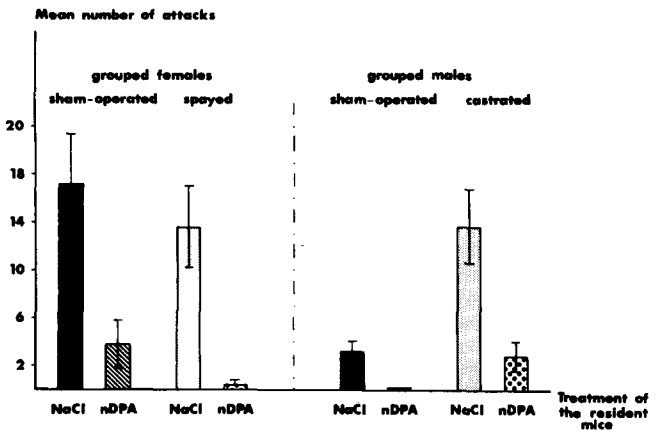

FIG. 1. Mean number of attacks directed by groups of intact and gonadectomized male and female mice toward strange lactating females.

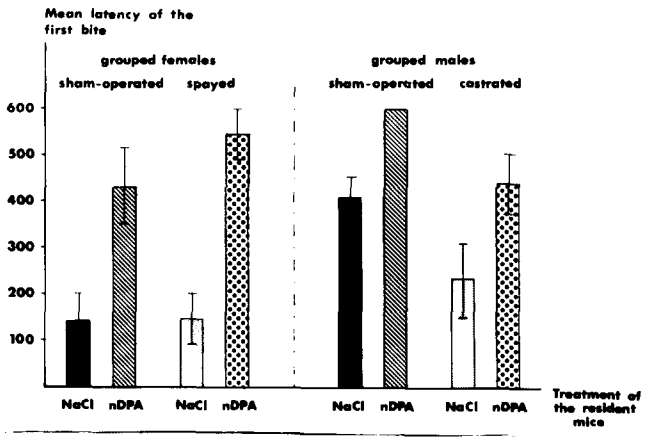

FIG. 2. Mean latency of attacks directed by groups of intact and gonadectomized male and female mice toward strange lactating females.

TABLE 2

GABA LEVELS IN DIFFERENT MOUSE BRAIN AREAS AFTER SALINE OR DPA/200 mg/kg (IP) TREATMENT IN SHAM-OPERATED AND GONADECTOMIZED MALE AND FEMALE GROUPED MICE

	Hypothalamus	Olfactory bulbs	Amygdala	Frontal cortex	Anterior colliculus	Posterior colliculus
Intact male						
+ NaCl	5.05 ± 0.45 (5)	4.25 ± 0.40 (6)	3.00 ± 0.30 (7)	2.50 ± 0.55 (8)	5.45 ± 0.50 (6)	3.30 ± 0.30 (6)
+ DPA	7.75 ± 1.75* (14)	6.40 ± 1.40* (10)	5.10 ± 0.70* (10)	3.60 ± 1.30* (12)	6.15 ± 1.05 (9)	4.30 ± 0.45* (10)
Castrated male						
+ NaCl	6.40 ± 0.90 (9)	5.00 ± 0.50 (9)	3.85 ± 0.40 (9)	2.65 ± 0.30 (7)	4.45 ± 1.05 (8)	3.10 ± 0.45 (9)
+ DPA	9.10 ± 1.50* (9)	6.55 ± 1.70* (8)	4.90 ± 1.30* (9)	3.30 ± 0.85* (10)	5.30 ± 1.95 (8)	5.30 ± 2.30* (7)
Intact female						
+ NaCl	6.20 ± 0.75 (9)	3.80 ± 0.70 (9)	3.30 ± 0.20 (9)	2.75 ± 0.45 (5)	4.55 ± 0.50 (6)	3.55 ± 0.55 (6)
+ DPA	9.50 ± 2.85* (9)	5.05 ± 1.00* (7)	4.10 ± 0.60* (8)	4.35 ± 1.60* (12)	6.35 ± 2.05* (7)	4.00 ± 0.65 (8)
Spayed female						
+ NaCl	6.40 ± 0.75 (6)	4.75 ± 0.70 (9)	3.40 ± 0.50 (5)	2.45 ± 0.45 (9)	5.15 ± 0.60 (10)	3.50 ± 0.25 (8)
+ DPA	9.60 ± 1.80* (8)	4.85 ± 0.80 (5)	4.35 ± 0.55* (5)	3.15 ± 0.95* (11)	7.10 ± 2.10* (8)	6.50 ± 2.20* (8)

GABA level is expressed in μmole/g microwaved treated tissue ± SD.
The number of animals is indicated between parentheses.
Significance: *$p<0.05$.

cages; i.e., 80 lactating females were used as "stimulus animals". Each behavioral test was performed 10 min after DPA or NaCl injection. When no attacks occurred in a 10-min period, a latency of 600 sec was assigned.

Biochemical Determination of GABA

Animals in each treatment category were killed immediately after the 20 min period by microwave treatment which inactivated the enzymes responsible for the post-mortem increase in GABA level.

After decapitation, the brain was removed from the skull and the hypothalamus, amygdala, olfactory bulbs, posterior and anterior colliculus and frontal cortex were dissected and stored at −25°C. Extraction and determination of GABA were performed according to the dansylation method [24]. The stable dansyl derivative was separated from other compounds by one-dimensional chromatography on silica gel[G]

layers and can be quantitatively determined by direct fluorometry of the thinlayer plates in the range of 0.1 to 5 n-moles/spot on an Aminco-Bowman spectrophotofluorimeter.

RESULTS

Effects of DPA on Aggression in Mice

Mean scores for various treatment are illustrated in Figs. 1 and 2. Data were treated by an analysis of variance and the Newman-Keuls method [16]. These analyses revealed that the amount of aggression toward lactating strangers was significantly affected by the factor "treatment", $F(1,72)=49.36$ (attacks); $F(1,72)=30.00$ (latency): $p<0.001$, the lowest incidence of aggressive responses occurring in both resident groups after DPA injections. A significant interaction was evident between the sex of the resident mice and its hormonal condition, $F(1,72)=7.47$ for attacks and $F(1,72)=9.44$ for latency: $p<0.01$ in both cases. This interaction was due to the increased level of aggression of the castrated males relative to the intact ones.

Application of the Newman-Keuls method confirmed that DPA treated resident groups of mice were less aggressive (usually $p<0.01$ for both criteria) towards lactating strangers with respect to NaCl controls of both sexes (except intact males).

Effects of DPA on GABA Content of Various Brain Areas

The results are summarized in Table 2. Comparisons among the different categories of results were usually made with a modified t-test [16].

Comparable elevations (40–50%) of hypothalamic GABA were observed after DPA in intact and gonadectomized male and female mice. The greatest increase in the GABA content of olfactory bulbs (50%) was evident in intact male mice. Identical elevations of GABA were observed in castrated males and in intact females but, in this neural area, the level of GABA remained unchanged in DPA-treated spayed females. The highest increase of amygdaloid GABA (70%) was measured in intact males. In other categories of animals this elevation reached only 25 to 30%. In the anterior colliculus, the increase in intact and castrated, DPA-treated males did not differ from their controls but (in this area) DPA did increase GABA concentration (40%) in intact and spayed grouped females. GABA increases in the posterior colliculus were highest in both gonadectomized male (70%) and female (85%) mice. The increase in GABA content after DPA treatment was smaller in intact males and females; c.f. posterior colliculus of gonadectomized animals. GABA level increased in the frontal cortex to a greater extent in intact males (45%) and females (60%) than in gonadectomized animals of both sexes (about 25%).

DISCUSSION

These results largely confirm and extend our previous findings [10,12]. Gonadal oestrogens do not influence this type of aggressive response by recipient female mice to lactating intruders. In this test situation, however, gonadal androgenic stimulation generally "inhibits" male aggression.

It was clearly demonstrated in Experiment 2 that after castration, aggressive responses displayed by recipient males against lactating intruders were comparable to those of intact or spayed females. This effect can be reversed by postcastrational steroid hormone treatment [12]. Very low levels (in intact and spayed females as well as in castrated males) or a total disappearance (intact males) of attack on lactating intruders occurred after treatment with DPA. This suppressed fighting could be correlated with an increase of GABA level in most of the brain areas studied. This inverse relationship between GABA and aggressiveness is reminiscent of comparable studies on muricidal aggression [18] and spontaneous fighting in mice [2,3].

However, the rate increase of neural GABA after DPA treatment differed in gonadectomized and intact animals. In the frontal cortex, there was a smaller increase in GABA concentration of gonadectomized male and female mice than in intact animals. In the amygdala, there was also a smaller increase in GABA level in castrated compared to intact male mice. On the other hand, the DPA dose employed did not elevate the GABA level in the olfactory bulbs of spayed females, and of the anterior colliculus of intact and castrated males. Only the posterior colliculus showed a higher GABA increase in gonadectomized animals; c.f., intact counterparts.

It would be meaningless at the present time to speculate about whether the increase of GABA in one particular brain area is more significantly implicated than that of another brain area in the inhibition of this type of aggression. Some data obtained on other forms of aggression have suggested that the level of the neurotransmitter metabolism is usually more altered in the olfactory bulbs, hypothalamus and amygdala of rats and mice. But, extreme caution should be employed in extrapolating between these forms of aggression and the model used in the present study. In spayed females, for example, where DPA produced a decrease of the aggressive responses towards lactating intruders, the level of GABA in the olfactory bulbs was similar to that of untreated animals. Our future studies of this nature may generate better neurochemical correlates of this behavioral model. It seems important, for example, to compare the results with those of grouped intact and gonadectomized mice which have not been placed in contact with lactating intruders; i.e. in which performance of aggressive responses cannot account for the change. It might also be useful to investigate the direct effect of gonadectomy on GABA level in the different brain areas, and to extend these assays to the striatum and septum, structures in which decreased GABA levels have been found following castration [3].

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