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Brain Catecholamines, Spontaneous Bioelectrical Activity and Aggressive Behavior in Ants (*Formica rufa*)

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KOSTOWSKI, W., B. TARCHALSKA AND B. WAŃCHOWICZ. *Brain catecholamines, spontaneous bioelectrical activity and aggressive behavior in ants* (Formica rufa). PHARMAC. BIOCHEM. BEHAV. 3(3) 337–342, 1975. – The effects of dopamine (DA), 1-DOPA, diethyldithiocarbamate (DDTC) and haloperidol on aggressive behavior and spontaneous bioelectrical activity of the ant *(Formica rufa)* were studied. Drugs such as DA, 1-DOPA and DDTC increased mutual aggressivity in ants while it failed to change aggression directed towards other species of insects (e.g., the beetle *Geotrupes sp.*). The amplitude of EEG waves and the amplitude of neuronal discharges within the protocerebrum decreased after administration of both DA and 1-DOPA. Both DDTC and 1-DOPA increased the concentration of adrenaline as well as DA in the brain of ants. Haloperidol decreased intrageneric aggressivity but caused no evident changes in both EEG pattern and neuronal discharges. The present study indicates that catecholamines are critically involved in the organization of aggressive behavior in ants.

Brain catecholamines   Aggressiveness in insects   Catecholamines and aggressiveness   Ants

IN insects, the occurrence of dopamine (3-hydroxytyramine, DA) has been known for long time. High concentrations of this amine exceeding these of adrenaline (A) and noradrenaline (NA) were found in the body of both puparium and of the imago of some species [5, 14, 15, 16]. Using spectrofluorimetric method Frontali [8] has detected high concentration of DA in the protocerebrum of the cockroach *Periplaneta americana*. The physiological role of DA in function of the nervous system of insects is not established. It is known, that DA is a precursor of N-acetyldopamine, a compound involved in tanning of the cuticle [5]. In larvae, tyrosine could be converted to tyramine and then to N-acetyltyramine while in older larvae tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) and then decarboxylated to DA. Small amounts of DA are converted to dihydroxyphenylacetic acid [5,17]. The formation of noradrenaline and adrenaline was not extensively studied but it is known that noradrenaline can be formed in insects from DA [5]. It was recently demonstrated [16] that DA can influence a spontaneous activity of neurons in the insect brain. This amine was shown to inhibit the spontaneously active neurones in the protocerebrum of the ant *Formica lugubris Zett.* This inhibition started immediately after microelectrophoresis and was more progressive than in vertebrate neurones.

In this paper we have assessed the influence of DA and of some drugs such as DOPA, diethyldithiocarbamate (DDTC) and haloperidol on the spontaneous bioelectrical activity of the brain as well as on the aggressive behavior of the ant *Formica rufa*. All drugs used in this study are known to influence brain DA. DOPA is an immediate precursor of DA and parenteral administration of this compound leads to increase in DA concentration in brain [10,11]. DDTC is a potent inhibitor of dopamine hydroxylase and increases DA concentration in the brain [9]. On the other hand haloperidol, the strong neuroleptic drug, is known to block the receptors for DA and increases the turnover of this amine [2,3]. The present study is an extension of previous works from our laboratory dealing with the action of some neurohormones and psychotropic drugs on the brain of the ants.

METHOD

Aggressiveness in Ants

The modified test of ants attacking the beetle *(Geotrupes sp.)* was used (for details see [13]). Ten–12 ants were placed into a specially prepared Petri dish surrounded with water and after 15 min, necessary for adaptation of the ants, the beetle was placed in the dish. The

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number of ants attacking the beetle and ants fighting among themselves were counted as separate groups. Drugs were dissolved in 0.6 percent NaCl and injected into the abdominal cavity through a thin glass cannula connected with a microsyringe. Control groups were given saline. The volume of solutions injected was 4–5 μl. Immediately after injection ants were placed into special plastic box and then tested for aggressiveness after consecutive 30 min, 1 hr, 2 hr and 3 hr. Some groups of ants were examined for brain catecholamine concentrations as described below. For statistical analysis a chi-square test was used [1]; data were presented in percentage values in respect to number of insects tested in each group.

Spontaneous Electrical Activity of the Brain in the Ants

The details of method were described in our previous papers [12,13]. The electroencephalographic activity (EEG) from the protocerebrum (right and left optic lobes) of the ant was recorded using tungsten wire electrodes 50–80 μm in dia. and insulated by epoxide varnish. The EEG records were made with an EEG Biofizpribor apparatus (USSR). For recording the neuronal discharges from optic lobes (multiunit activity, MUA) electrodes were made of stainless steel wire electrolytically sharpened (15–20 μm at the tips) insulated by epoxide varnish. Electrodes were placed on the exposed surface of the optic lobe or lowered 10–20 μm into the lobe by means of a micromanipulator. Both EEG and MUA activity were recorded from bipolar electrodes. The MUA activity was displayed on the Cossor oscilloscope screen and photographed. Drugs were dissolved in 0.6 percent saline and applied locally on the exposed brain or injected into the abdominal cavity in amounts of 4–5 μl.

Estimation of Brain Catecholamines

Brains were removed under careful microscopic control and homogenized in 2 ml of 0.4N perchloric acid and subsequently analyzed for catecholamines according to fluorimetric method described by Chang [4]. A Beckman fluorimeter was used. Twenty brains were collected for one sample.

Drugs

The drugs used were: dopamine (3-hydroxytyramine, Fluka), l-DOPA (3,4-l-dihydroxyphenylalanine, Fluka), diethyldithiocarbamate (Eastman Organic Chemicals) and haloperidol (Janssen).

RESULTS

Aggressiveness in Ants

The percentage of ants attacking the beetle in the control group injected with saline was within 39–51 percent. The aggressiveness was significantly lower 3 hr after injection of saline as compared with that measured 30 min after administration of saline. The percentage of ants showing intrageneric aggression (fighting among themselves) never exceeded 14 percent. This value was significantly lower 1 hr and 2 hr after injection of saline as compared with values observed 30 min following the administration of NaCl. These results are summarized in Fig. 1.

l-DOPA (0.3–0.6 μg/mg) injected into the abdominal cavity caused changes in general behavior of ants such as ataxia and increase in motor activity. The last effect was only visually observed. l-DOPA markedly increased the percentage of ants fighting among themselves (up to 30 percent), the maximal effects were observed 1–3 hr after administration of this drug. On the other hand, no changes in number of ants attacking the beetle were practically observed. DA injected into the abdominal cavity at the doses of 0.2–0.3 μg per mg like l-DOPA increased the number of ants showing intrageneric aggression but caused no changes in the number of ants attacking the beetle. The maximal effect was observed 30 min after administration of DA (Figs. 2, 3).

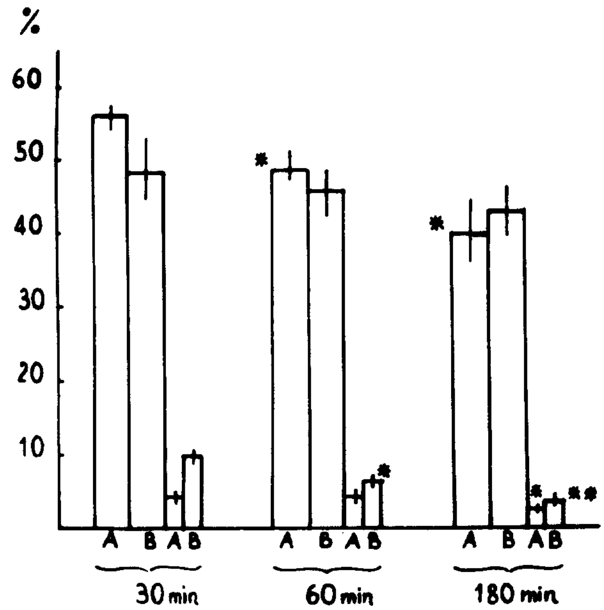

FIG. 1. Aggressiveness of control ants receiving saline. Large columns – attacking the beetle (intergeneric aggressiveness, small columns – mutual aggressiveness (intrageneric aggressiveness. Ordinate – aggressiveness in percent; abscissa – time after injection of the saline. A 2 min and B 5 min after starting the test. Vertical bars: confidence limits at 0.5% level for percentage values. *$p<0.05$, **$p<0.01$ as compared with 30 min.

DDTC (0.3–0.6 μg/mg) caused motor disturbances in ants such as ataxia, tremor of extremities and circulatory movements. This drug tremendously increased the number of ants fighting among themselves (39–47 percent). This intrageneric aggressivity was higher than intergeneric aggressivity, i.e., aggressivity directed toward the beetle (Fig. 4). The later aggressivity was even significantly decreased 2 hr after injection of DDTC. Haloperidol (0.3–0.6 μg/mg) caused opposite effects on aggressiveness to other drugs used in these experiments. One hour after administration, the number of ants attacking the beetle significantly increased while the number of ants fighting among themselves decreased (48–52 percent versus 0–4 percent, see Fig. 5). Haloperidol decreased the general motor activity but caused no abnormal movements in ants.

Spontaneous Electrical Activity of the Brain of the Ant

The EEG pattern of the protocerebrum of the ant consisted of waves of 10–50 μV with a frequency of

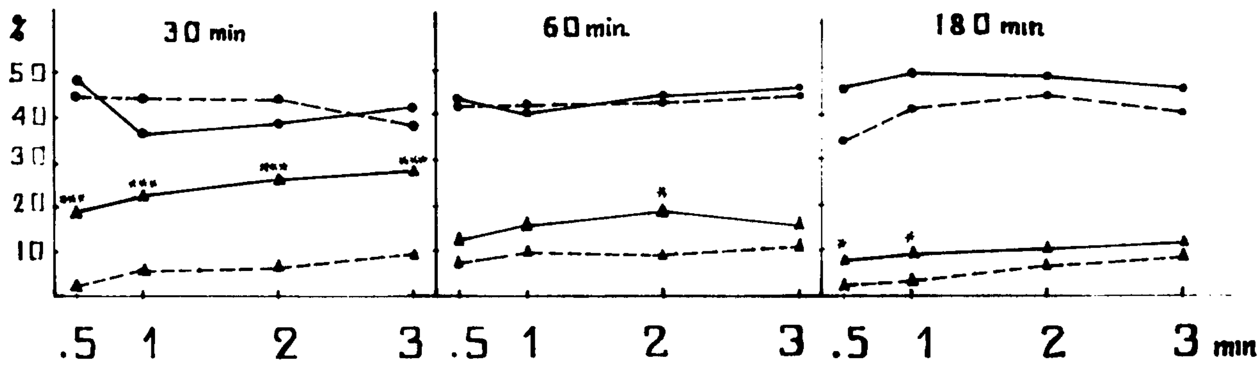

FIG. 2. Effects of dopamine on aggressive behavior in ants. Ordinate – aggressiveness in percent, time after starting the test marked in abscissa (30 sec, 1 min, 2, 3 and 5 min). ● – attacking the beetle, ▲ – mutual aggressivity. Dashed lines indicate aggressivity of control ants, the full lines indicate aggressivity of treated ants. The time after injection of dopamine marked in the figure.

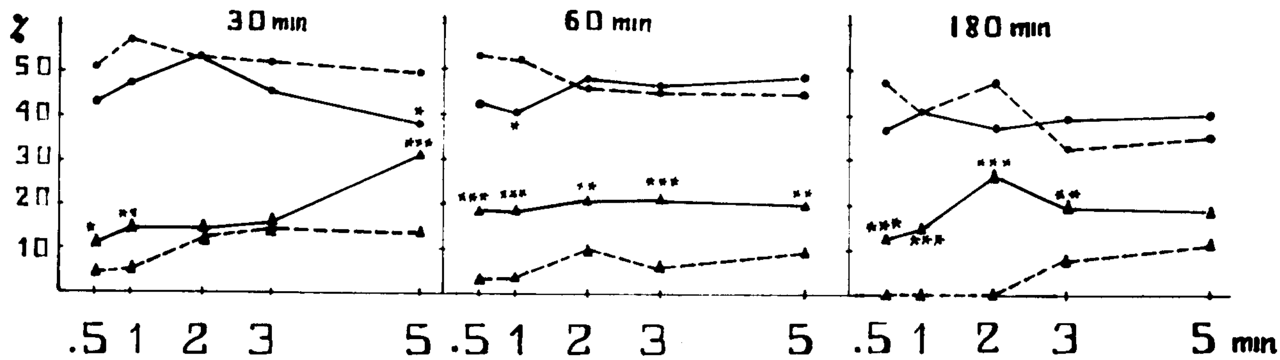

FIG. 3. Effects of l-DOPA on aggressive behavior in ants. Explanations as in Fig. 2.

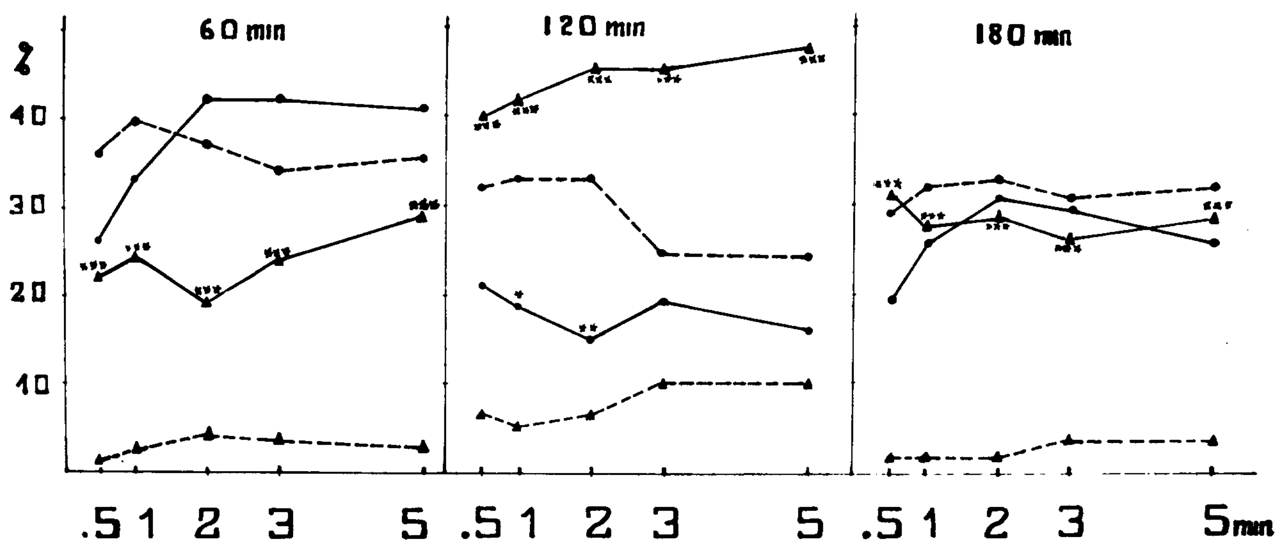

FIG. 4. Effects of diethyldithiocarbamate on aggressive behavior on aggressive behavior in ants. Note enormous increase in intrageneric aggressivity. Explanations as in Fig. 2.

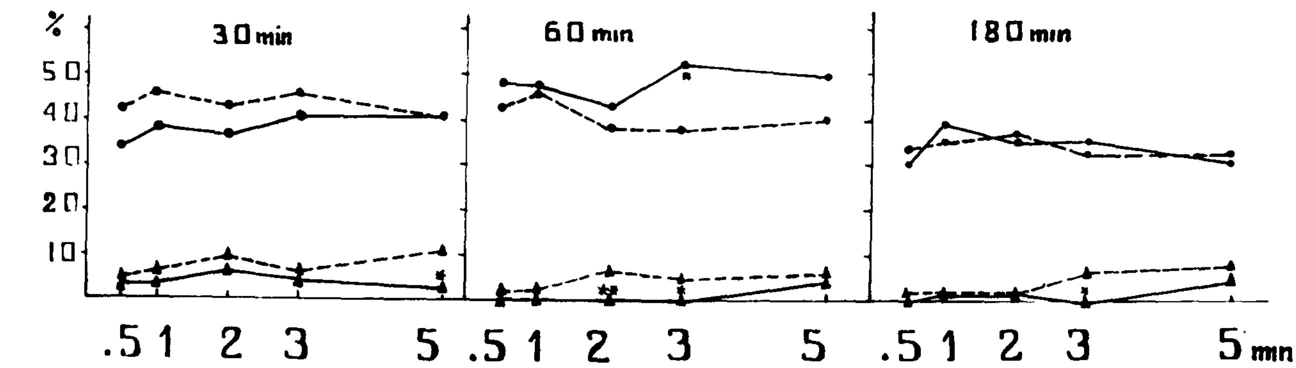

FIG. 5. Effects of haloperidol on aggressiveness in ants. Explanations as in Fig. 2.

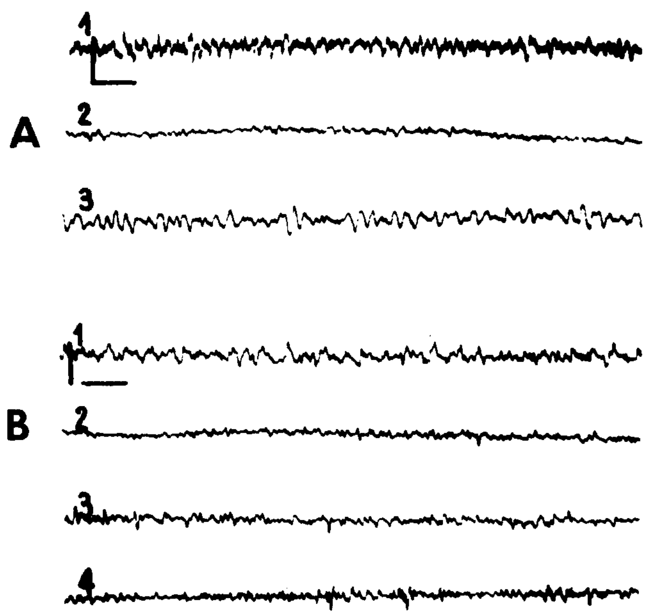

FIG. 6. EEG of ant. (A) Effect of DA applied locally on the exposed brain. (1) Control pattern, (2) 1 min, (3) 10 min after application of DA. (B) Effect of DA (0.5 μg/mg) injected into the abdominal cavity. (1) Control pattern, (2) 5 min, (3) 10 min and (4) 15 min after injection. Vertical bar – 100 μV, horizontal – 1 sec.

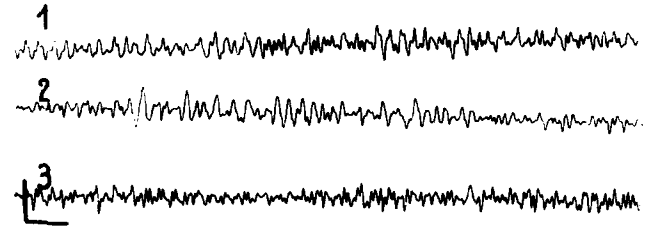

FIG. 7. EEG of ant. Effect of l-DOPA (0.5 μg/mg) injected into the abdominal cavity. (1) Control pattern, (2) 5 min and (3) 20 min after injection. Calibration 100 μV and 1 sec.

3–8/sec. The MUA pattern recorded from optic lobes consisted of more or less regular bursts of spikes of 10–20/sec and of amplitude 20–100 μV. DA injected into the abdominal cavity (0.5 μg/mg) or applied locally on the exposed brain (1.10^{-4} g per ml) decreased the amplitude of EEG waves but did not change their frequency. The maximal effect was observed 15–30 min after administration of this drug (6 experiments). Both the amplitude and the frequency of neuronal discharges recorded from the optic lobes markedly decreased after the local application or intraabdominal injection of DA. This effect started almost immediately after the local application of DA (1.10^{-4} g per ml), and lasted for 1 hr or more (Figs. 6, 8). l-DOPA (0.5 μg/mg injected into the abdominal cavity likewise DA decreased the amplitude of EEG waves (6 experiments) and inhibited neuronal firing within the optic lobes (4 experiments). The effects of l-DOPA were, however, less marked than these of DA and started 2–4 min after administration (Figs. 7, 9). Haloperidol injected into the abdominal cavity (0.5 μg/mg) did not influence both the EEG pattern and MUA pattern (6 and 4 experiments, respectively).

Brain Catecholamines Concentration after Administration of l-DOPA and DDTC

The levels of A, NA and DA in control ants, examined 2 hr after the administration of 0.6 percent NaCl were 2.92 ± 0.18 μg/g, 8.09 ± 0.42 μg/g and 9.48 ± 1.01 μg/g, respectively. l-DOPA injected into the abdominal cavity (0.5 μg/mg markedly increased concentrations of both DA and A. DDTC (0.5 μg/mg) slightly and not significantly increased DA concentration in the brain whilst significantly decreased the level of NA. The level of A was slightly but significantly increased (see Table 1).

DISCUSSION

The present study indicates that drugs that change concentrations of catecholamines in the brain such as l-DOPA and DDTC increase intrageneric aggressiveness in ants. The most marked effects were observed after the administration of DDTC. This drug decreased brain NA level while it increased both A and DA concentrations. l-DOPA led to a marked increase in the DA and A level and insignificantly elevated brain NA. Our observations suggest therefore that increase in brain DA and A concentrations is

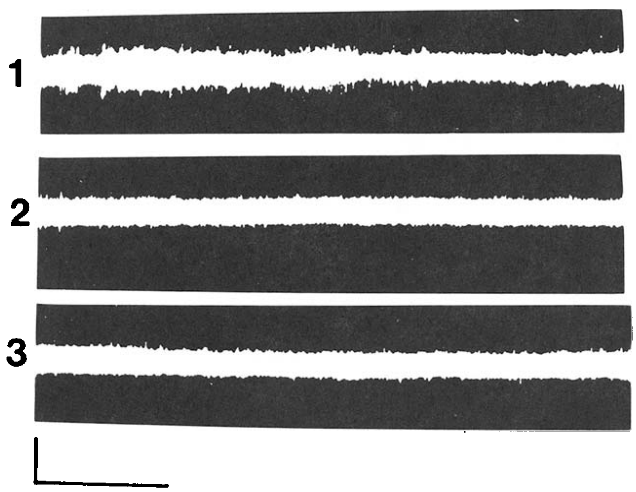

FIG. 8. Multi-unit activity recorded from optic lobe of the ant. Effect of DA applied locally on the brain. (1) Control pattern, (2) 2 min and (3) 15 min after application of DA. Vertical bar – 100 μV, horizontal – 1.5 sec.

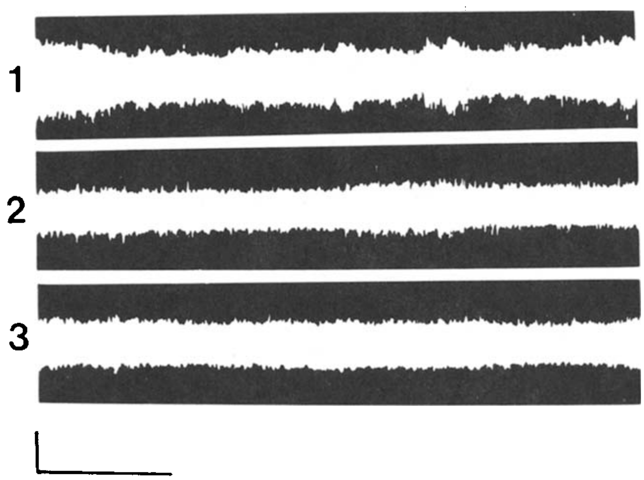

FIG. 9. Multi-unit activity recorded from optic lobe of the ant. Effect of l-DOPA injected into the abdominal cavity (0.5 μg/mg). (1) Control pattern, (2) 5 min and (3) 30 min after l-DOPA. Calibration 100 μV and 1.5 sec.

TABLE 1

THE EFFECTS OF l-DOPA AND DIETHYLDITHIOCARBAMATE (DDTC) OF BRAIN CATECHOLAMINES CONCENTRATION IN ANTS *FORMICA RUFA* *

Treatment	Brain Content μg/g (mean ± S.E.M.)		
	Adrenaline	Noradrenaline	Dopamine
Control†	2.92 ± 0.18 (12)	8.09 ± 0.42 (15)	9.48 ± 1.01 (12)
l-DOPA‡	6.82 ± 0.68^{c} (9)	10.07 ± 1.00 (9) N.S.	26.77 ± 2.74^{c} (7)
DDTC§	3.88 ± 0.32^{b} (10)	6.51 ± 0.42^{a} (10)	13.31 ± 2.36 (12) N.S.

*Figures in parentheses indicate number of experiments.

†Controls received 0.5 μl of 0.6% saline and were examined 2 hr after the administration of saline.

‡l-DOPA given at the dose of 0.5 μg/mg, ants were examined 3 hr after the administration.

§Ants examined 2 hr after the administration of 0.5 μg/g of DDTC.

$^{a}p<0.05$, $^{b}p<0.02$, $^{c}p<0.001$ for difference from control (Students t-test)

NS – not significant

critical for development of mutual aggressivity in ants. The role of NA seems to be less clear in this behavior but it should be mentioned that DDTC, the compound producing the most marked effect on intrageneric aggressiveness, led to decreases in the level of this amine. The increase of A concentration after DDTC administration with a concomitant reduction of brain NA is difficult to explain. It is possible to hypothesize the existence of different pathways of NA and A biosynthesis in the ant brain but other explanations are also possible. The increase in both DA and A levels after 1-DOPA administration indicates that this amino acid could be transformed into DA and subsequently A in the brain of ants. It may be supposed that effects of DA and 1-DOPA as well as DDTC on aggressiveness in ants are related with specific catecholaminergic mechanisms since haloperidol, the drug blocking receptors for DA and NA causes opposite effects. The different effects of 1-DOPA and DDTC on aggressivensss may be connected with various influences on brain NA level. One point to be considered is that using the method of Chang [4] not only catecholamines are determined but also DOPA. It is therefore not possible to make definite statements about the precise values of concentrations of these substances in the brain of the ant.

Both DA and 1-DOPA decreased the amplitude of EEG waves as well as reduced the spontaneous activity of neurones within the protocerebrum of ants. Our findings are in accordance with these of Steiner and Pieri [16] indicating that DA produced clear-cut inhibition of spontaneously active neurones in the brain of the ant *Formica lugubris Zett*. It cannot, however, be decided at present whether these changes are related to behavioral effects of DA and 1-DOPA. Since behavioral motivation of ants genus *Formica* is based to a great extent on visual perception [6,7] we may suppose that changes in the activity of neurones within the optic lobes would be responsible for some behavioral phenomena.

The present study provides the evidence that in insects such as ants, genus *Formica*, catecholamines are critically involved in the organization of aggressive behavior. This problem, however, requires further elucidation.

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