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BRIEF COMMUNICATION

Aggressive Behavior and Brain Serotonin and Catecholamines in Ants (*Formica rufa*)

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KOSTOWSKI, W., B. TARCHALSKA-KRYNSKA AND L. MARKOWSKA. *Aggressive behavior and brain serotonin and catecholamines in ants* (Formica rufa). PHARMAC. BIOCHEM. BEHAV. 3(4) 717–719, 1975. – Serotonin, adrenaline and noradrenaline were analyzed in brains of the ants, *Formica rufa*. Experiments demonstrated that concentrations of both serotonin and adrenaline were higher in ants that displayed aggressiveness (interspecific and intrageneric) while concentrations of noradrenaline was decreased.

Aggressiveness 5-HT Catecholamines Brain monoamines in ants

ANTS are insects showing a natural aggressiveness directed towards other insects (i.e. interspecific or intergeneric aggressivity). Our previous studies indicated that some biogenic amines and their precursors influenced this aggressive behavior and in some circumstances induced mutual (intrageneric) aggressiveness in ants [5, 6, 7, 8]. Both serotonin (5-HT) and 5-hydroxytryptophan decreased aggressiveness directed towards the beetle [8]. On the other hand dopamine (DA) and 3,4-dihydroxyphenylalanine (L-DOPA) caused no clear effects. Increased mutual aggressiveness in ants have been observed after the injections of all compounds mentioned above as well as after the injection of diethyldithiocarbamate, the inhibitor of DA hydroxylase [10]. The objective of the present study was to describe the effects of aggressiveness on brain 5-HT and catecholamines concentration in ants, *Formica rufa*.

METHOD

Interspecific aggressiveness was induced using the test of ants attacking the bettle [5,8]. Ants were kept in a special plastic formicarium. For experiments 10–12 ants were placed in a Petri dish 10 cm in dia. surrounded with water and after a 15 min acclimatization a beetle (*Geotrupes sp.*) was placed in the same dish amont them. Ants were allowed to attack the beetle for 15 min and then the beetle was removed. Sixty min after the attack ants were decapitated, their brains were quickly removed under microscopic examination and immediately homogenized with 0.4 N perchloric acid (for estimation of 5-HT ascorbinic acid, sodium metabisulfide and disodium ethylendiaminetetraacetate, EDTA were added) and subsequently analyzed for catecholamines and 5-HT. Sixty min was chosen since maximal changes in brain 5-HT were observed previously by us [9] after this period in ants attacking the beetle. Intraspecific aggressiveness was induced in ants by cutting the antennae as described by Vowles [15]. After cutting the antennae 10 ants were placed in a Petri dish for 60 min. During this period ants attacked one another and then were decapitated and subsequently analyzed biochemically. For determination of 5-HT the fluorimetric method of Bertler [1] was used; catecholamines were measured according to Chang [2]. Twenty brains were used for each sample for estimation of catecholamines and 50 brains used for 5-HT.

RESULTS

The results summarized in Table 1 show that concentrations of 5-HT and catecholamines were altered in ants after the aggressive display. The most pronounced effect

TABLE 1

BRAIN 5-HT AND CATECHOLAMINES LEVELS IN ANTS FORMICA RUFA DISPLAYING AGGRESSIVE BEHAVIOUR

Experimental Conditions	Content in Brain (μg/g) Serotonin	Adrenaline	Noradrenaline
Control 1	6.47 ± 0.25 (8)	2.80 ± 0.33 (17)	7.92 ± 0.46 (12)
Control 2	7.62 ± 0.32* (7)	3.37 ± 0.34 (7)	6.95 ± 1.00 (7)
Interspecific aggressiveness	9.99 ± 0.49‡b (7)	4.79 ± 0.86* (9)	4.96 ± 1.06† (6)
Interspecific aggressiveness	9.05 ± 0.80‡a (3)	5.97 ± 0.87‡§ (8)	5.36 ± 1.21* (6)

Number of samples in brackets (20 brains were used for each sample for estimation of catecholamines and 50 brains used for each sample for estimation of 5-HT). Control 1 – ants taken directly from the formicarium, Control 2 – ants isolated for 60 min prior to the examination (see text for details). Means ± SE.

* Differs from Control 1 mean at $p<0.05$
† Differs from Control 1 mean at $p<0.02$
‡ Differs from Control 1 mean at $p<0.01$
§ Differs from Control 2 mean at $p<0.05$
[a] Differs from Control 2 mean at $p<0.02$
[b] Differs from Control 2 mean at $p<0.01$

was an increase of 5-HT concentration in brain of ants displaying aggressiveness of both types: interspecific (against the beetle) and intraspecific (mutual). It should be noted that small but statistically significant increase in brain 5-HT concentration was also observed in ants isolated for 60 min out of the formicarium as compared with ants examined biochemically immediately after being taken out of their nest. Concentration of 5-HT in ants following the attack was, however significantly higher as compared with non-isolated control groups as well as isolated ones (in Table 1 designated as Control 1 and Control 2 respectively). Concentrations of adrenaline were also higher in brains of ants that displayed aggressiveness whereas noradrenaline level was reduced in these animals. Isolation caused only insignificant changes in the levels of both noradrenaline and adrenaline in brains. These trends however were toward the same directions as those induced by aggressiveness. Changes in the levels of catecholamines in brain of aggressive ants were significant only as compared with control group 1 (i.e. non-isolated out of the formicarium) except for ants displaying mutual aggressiveness and examined for adrenaline concentration.

DISCUSSION

Previous studies from our laboratory have shown by use of paper chromatography that ants have higher brain 5-HT levels after attack [9]. No quantitative analyses, however, have been made previously. The results of this study confirm these findings and indicate that a decrease in brain noradrenaline concentration and an increase in adrenaline concentration parallel the changes in 5-HT level. The mechanisms of these phenomena remain, however, unexplained. It may reflect the interaction between 5-HT and catecholamines since Johnson and Kim have reported recently [4] that inhibition of DA hydroxylase leads to an increase of brain levels of tryptophan in rats. It is also possible that this differential effect on adrenaline and noradrenaline levels after fighting is due to alteration in turnover and/or synthesis of adrenaline.

It is worth mentioning that separation of ants from their nest causes similar (but less marked) changes to those observed after the attack. It may be, therefore, concluded that these changes are, at least in part, due to nonspecific stress accompanying the isolation. In higher animals such as rodents, increase in brain 5-HT accompanies stress (acute exposure to cold, restrain stress) [16]. Fighting episodes also cause increases in brain 5-HT level in mice [16,17]. On the other hand some authors have postulated that mice and rats show a decreased turnover in brain 5-HT which is accompanied by aggressiveness induced by prolonged isolation [13,14].

Turning to our earlier reported finding that 5-HT and 5-HTP decrease interspecific aggressiveness in ants [8,9] it

is difficult to reconcile these data with the present finding that 5-HT apparently increases with aggression. In our previous studies, however, 5-HT and its precursor were given 30 min – 3 hr before ants were tested and no relation between brain 5-HT and the fighting episode itself was analyzed. It should be also noted that both 5-HT and 5-HTP increased intraspecfic (mutual) aggressiveness in ants [8,9]. The possible changes in levels and turnover of brain catecholamines in aggressive laboratory animals have been reported by some authors [3, 11, 12].

From the present experiments it would seem that changes in brain catecholamines and 5-HT are, similarly to higher animals, involved in aggressive behavior in insects such as ants genus Formica. This response may be mediated by changes of intermediate hormonal messengers since these animals may have been secreted more phenomones, defense agents and other substances. Further studies are obviously needed to explain above described phenomena.

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