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Biochemical correlates of agonistic behaviour in *Bandicota bengalensis*: Hepatic cholesterol and ascorbic acid

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Abstract. Social stress was induced in *Bandicota bengalensis* by staging 10 min encounters in neutral area between male-male, male-female and residents- intruder male for 9 continuous days. On the 10th day changes in the weight, ascorbic acid, and cholesterol levels of liver were estimated. In submissive males, females of male-female encounter and intruders, adrenals hypertrophied. Liver weight remained unaltered. Ascorbic acid levels increased in both members of heterosexual pair, residents and intruder but decreased in subordinates compared to controls. Cholesterol increased in subordinate males and stressed females.

Keywords. Social stress; *Bandicota bengalensis*; adrenals; liver; ascorbic acid; cholesterol.

1. Introduction

Amongst small mammals increased adrencorticotrophic hormones (ACTH) are implicated in crowding and other social stressful situations leading to hyperadrenalism, increased adrenalin and noradrenalin content of the adrenal glands and enhanced secretion of adrenal steroids (Barnett 1969). Increased steroid production by adrenals involves cholesterol as substrate and the reaction is dependent on ascorbic acid. Both cholesterol and ascorbic acid are synthesized in the liver and hence the levels of these can be considered as an index and reflection of social stress in rodents. Archer (1969) regarded adrenal cholesterol and ascorbic acid levels to reflect crowding stress in mice. This paper examines the significance of changes in hepatic cholesterol and ascorbic acid content during social stress in *Bandicota bengalensis*.

Based on inter-species conflict *B. bengalensis* is considered as the most aggressive wild rodent in India (Spillet 1968; Sridhara *et al* 1980). The species also exhibits high levels of intraspecies strife (Sridhara and Krishnamoorthy 1983). Adrenal hypertrophy was shown to accompany such interspecific agonistic interactions (Sridhara *et al* 1983). The present paper reports hepatic levels of cholesterol, the precursor for adrenal steroid synthesis and ascorbic acid levels in *B. bengalensis* exposed to different kinds of social stress.

2. Materials and methods

Wild *B. bengalensis* were collected and maintained according to Sridhara and Krishnamoorthy (1983). Behavioural stress was induced by three ways (i) by allowing

interactions between two adult males for 15 min daily (ii) by staging male-female encounters for 15 min daily (iii) by introducing an intruder male into an all male resident cage for the same duration daily. The paired interactions were staged in a behaviour chamber described elsewhere (Sridhara and Krishnamoorthy 1983). It consisted of a 100 × 50 × 50 cm galvanized iron chamber with a glass front, glass sides of sliding type to facilitate introduction of animals and a wiremesh top. A slot in the centre of the roof enabled insertion of a thin metal sheet thus dividing the chamber into two equal portions.

Prior to induction of stress the animals were kept in isolation for 15 days. From days 16–24 members of hetero- and iso-sexual pairs were introduced into either side of the behaviour chamber and allowed to habituate to the test environment for 10 min. Later the metal partition was removed and the two animals allowed to interact for 10 min daily over a nine day schedule. Similarly three adult males of differing weights were kept in a single cage (35 × 35 × 50 cm) for 15 days. Such animals were considered residents in contrast to single males kept in isolation. The latter were assigned intruder status as they were put into resident cages from 16th to 24th days for 10 min each day for 9 continuous days.

Rats of both sexes, kept in isolation served as controls.

On the 25th day both experimental and control animals were weighed and sacrificed by decapitation. Adrenals, both left and right and whole liver were excised, cleaned of blood and weighed. The levels of ascorbic acid, and cholesterol from livers of differentially stressed rats were determined according to Omaye *et al* (1979) and Sperry and Webb (1950) respectively.

Adrenal: body weight and liver: body weight ratios were calculated and compared for different stress situations to see if hypo- or hypertrophy of the organs ensued consequent to social stress. A total of six male-male, six male-female and six resident-intruder interactions were staged, each over a nine day schedule. Statistical significance were arrived at by student t tests.

3. Results

The behavioural aspects of the three different social situations have been reported elsewhere (Sridhara *et al* 1983). Briefly one male dominated the other in male-male encounters. The latter was considered submissive. In heterosexual encounters males dominated females although females too exhibited aggressive behaviour. Residents were agonistic towards intruders.

Adrenals hypertrophied in submissives, females of male-female conflict and intruders (table 1). Females kept in isolation had heavier adrenals than their male counterparts. The sexes did not differ in the weight of liver. Similarly social stress did not affect liver weight in any of the experimental situations. Only submissive males and females of heterosexual conflict registered significantly lower hepatic ascorbic acid levels compared to dominants and males of male-female pair respectively (table 1). Compared to control males the liver ascorbic acid levels decreased only in subordinate rats ($P < 0{\cdot}01$) but rose considerably in males of heterosexual conflict ($P < 0{\cdot}01$) and in both residents ($P < 0{\cdot}05$) and intruders ($P < 0{\cdot}02$). Similarly stressed females had higher ascorbic acid levels compared to female controls ($P < 0{\cdot}01$).

Amongst controls the two sexes did not differ in cholesterol levels of liver (table 1).

Table 1. Changes in weight of adrenal, hepatic, ascorbic acid and cholesterol levels due to social stress.

	Control			male *vs* male			male *vs* female			resident *vs* intruder		
	M	F	$P<$	dom	sub	$P<$	M	F	$P<$	res	int.	$P<$
Body weight (g)	178	173		181	203		201	174		225	231	
	±	±		±	±		±	±		±	±	
	3·3	4·02	NS	6·26	3·12	NS	6·75	8·56	NS	7·52	3·83	NS
Adrenal weight (mg)	103·4	397·7		126·7	243·6		311·5	690·01		517·5	866·25	
	±	±		±	±		±	±		±	±	
	2·31	6·75	0·001	7·21	3·28	0·001	2·58	3·82	0·001	8·95	10·72	0·001
Adrenal wt/body wt	5·8*	23·0		7·00*	12·00		15·5	39·66		23·00	37·5	
(10^{-3} otherwise	±	±		±	±		±	±		±	±	
mentioned)	2·19	2·33	0·001	1·24	1·3	0·001	2·45	4·42	0·001	1·95	2·63	0·001
Ascorbic acid	12·7	13·64		13·33	6·66		28·15	22·03		23·04	24·94	
(mg/g)	±	±		±	±		±	±		±	±	
	1·07	2·07	NS	1·17	1·38	0·001	2·22	2·61	0·001	2·37	4·07	NS
Cholesterol (mg/g)	2·26	3·84		2·61	5·72		9·65	8·75		3·83	3·41	
	±	±		±	±		±	±		±	±	
	0·85	1·03	NS	0·23	0·7	0·001	0·94	0·36	NS	0·43	0·33	NS

* 10^{-4}.

NS: not significant.

Heterosexual and resident-intruder conflict did not affect cholesterol content of liver while male-male encounters resulted in enhanced levels in subordinate rats (table 1). Social stress elevated cholesterol levels in the liver of submissive males ($P < 0{\cdot}05$). Similarly females of heterosexual interaction had higher hepatic cholesterol levels compared to control females ($P < 0{\cdot}001$).

4. Discussion

Hyperactivity of adrenals and accompanying enhanced corticosteroid production has been correlated with density and territoriality (Christian and Davies 1964; Andrews *et al* 1972), disorganization of social behaviour (Benton *et al* 1978) and agonistic interactions (Bronson and Eleftheriou 1964; Archer 1969): Subordinate and defeated mice had heavier adrenals (Brain 1972; McKinney and Pasley 1979; Sridhara *et al* 1983). Adrenal hypertrophy was also reported in intruders and females of *Bandicota bengalensis* during resident-intruder and male-female confrontations respectively (Sridhara *et al* 1983). The elevated corticosteroid levels in such situations are believed to affect population dynamics in small mammals (Christian *et al* 1965). Cholesterol is the source of steroid hormones formed in adrenal cortex (White *et al* 1978). Increased social stress was correlated with depleted adrenal cholesterol during male-male, resident-intruder and male-female encounters (Sridhara *et al* 1983). Since liver is the major site of cholesterol synthesis, hepatic levels of cholesterol possibly reflect changed rates of corticosteroid synthesis. The present finding of higher hepatic cholesterol in submissive and female rats compared to dominants and males of heterosexual pairs lends credence to the theory of adrenal hyperactivity during social stress.

Barnett (1969) postulated that social interactions of mammals induces physiological changes which resemble those resulting from adverse conditions like cold or infection. Ascorbic acid content of adrenals, testis, liver and kidney of rats increased significantly during cold exposure (Dugal and Therien 1955). In several mammals ascorbic acid administration was shown to increase tolerance to cold acclimation and acclimatization (Lloyd and Sinclair 1953). Additionally ascorbic acid is essential for the synthesis of adrenal steroids from cholesterol. Liver is the site of ascorbic acid synthesis. In the current study the hepatic levels of ascorbic acid were higher in subordinates compared to dominants and females compared to males during male-male and male-female conflict respectively. Such enhanced synthesis of ascorbic acid may contribute towards coping with stress and also reflect increased demand for ascorbic acid due to elevated corticosteroid synthesis during behavioural stress.

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